2025 Laboratory Policies

MVP Health Care® policy and procedure guidelines.

Policies Effective April 1, 2025

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- Testing of Homocysteine Metabolism-Related Conditions
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- Urinary Tumor Markers For Bladder Cancer
- Urine Culture Testing for Bacteria
- Venous and Arterial Thrombosis Risk Testing
- Vitamin B12 and Methylmalonic Acid Testing
- Vitamin D Testing

Allergen Testing

Policy Number: AHS – G2031 – Allergen Testing	Prior Policy Name and Number, as applicable:
Initial Presentation Date: 09/18/2015 Revision date: 03/01/2024	

Policy Description

Allergic disease is characterized by inappropriate or exaggerated rated immune reactions to foreign antigens (allergens) that are generally innocuous to most people, but when introduced into a genetically-predisposed individual, elicit a hypersensitivity reaction (Hamilton, 2023). Hypersensitivity reactions can be classified into four types, two of which are associated with allergy, type I immediate immunoglobulin E (IgE) reactions and type IV T cell mediated reactions (Chang & Guarderas, 2018). Type I reactions involve the formation of IgE antibodies specific to the allergen. When the subject is re-exposed to that allergen, the allergen binds multiple IgE molecules, resulting in the release of an array of inflammatory mediators, including histamines, that precipitate the symptoms of allergic disease (Hamilton, 2023).

Allergen testing in serum is designed to detect the presence of allergen specific IgE. A positive test for allergen specific IgE confirms the presence of the antibody only. Actual reactivity must be determined by history or supervised challenge (Kowal & DuBuske, 2022). Several diagnostic procedures have been developed to elicit and assess hypersensitivity reactions including epicutaneous, intradermal, patch, bronchial, exercise, and ingestion challenge tests (Bernstein et al., 2008).

Related Policies

Policy Number	Policy Title
AHS – G2056	Diagnosis of Idiopathic Environmental Intolerance

Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) Specific IgE in-vitro allergy testing **MEETS COVERAGE CRITERIA** in **any** of the following situations:
   a) In lieu of skin testing for an **initial** allergy screen.
   b) When skin testing is contraindicated (see Note 1).
   c) When further treatment decisions would be impacted by confirmation of sensitivity in individuals for whom direct skin testing results are not consistent with the history of an anaphylactic or other severe reaction to an allergen.

2) When limited to allergens chosen for testing based on an individual's history, physical examination, and environment, specific IgE in-vitro allergy testing (up to 20 allergen specific antibodies per year) **MEETS COVERAGE CRITERIA**.

3) In-vitro testing for total serum IgE **MEETS COVERAGE CRITERIA** in **any** of the following situations:
   a) For individuals with moderate to severe asthma.
   b) For individuals with signs or symptoms of allergic bronchopulmonary aspergillosis.

4) To monitor for allergy resolution in children and adolescents with an initial positive food allergen result(s), annual re-testing for the same food allergen(s) **MEETS COVERAGE CRITERIA**.

5) In the absence of a new clinical presentation, routine re-testing for allergies to the same allergens (except where specified above) **DOES NOT MEET COVERAGE CRITERIA**.

6) The antigen leukocyte antibody test (ALCAT) **DOES NOT MEET COVERAGE CRITERIA**.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

7) For individuals with signs or symptoms of allergies, basophil activation flow cytometry testing (BAT) and in-vitro testing of IgG, IgA, IgM, **and/or** IgD **DO NOT MEET COVERAGE CRITERIA**.

8) In-vitro allergen testing using bead-based epitope assays (e.g., VeriMAP Peanut Dx) **DOES NOT MEET COVERAGE CRITERIA**.

9) For all situations, in-vitro testing using qualitative specific IgE multi-allergen screen that does not identify a specific allergen **DOES NOT MEET COVERAGE CRITERIA**.

NOTES:
Note 1: Skin testing is contraindicated in the following situations:

- Patients who have certain skin conditions (e.g., dermatographism, urticaria, cutaneous mastocytosis, atopic dermatitis, severe diffuse psoriasis)

- Patients who are taking medications that may interfere with the treatment of anaphylaxis (e.g., Beta-blockers and Angiotensin Converting Enzyme inhibitors) or may impair skin test sensitivity (e.g., tricyclic antidepressants, antihistamines)
- Patients who are at high risk to testing (e.g., poorly controlled asthma, clinical history of severe reaction to minute amounts of allergen, cardiac arrhythmia, unstable angina)
- Patients who have experienced an anaphylactic event within the past one month
- Uncooperative patients (e.g., small children, individuals with mental or physical impairments)

Table of Terminology

Term	Definition
AAAAI	The American Academy of Allergy, Asthma, and Immunology
AAAI	Academy of Allergy, Asthma, and Immunology
AAFP	American Academy of Family Physicians
AAP	American Academy of Pediatrics
ACAAI	American College of Allergy, Asthma, and Immunology
ACD	Allergic contact dermatitis
ACR	American cockroach
AIT	Allergen immunotherapy
ALCAT	The Antigen Leukocyte Antibody Test
AR	Allergic rhinitis
Arah2-sIgE	*Arachis hypogaea* 2-specific immunoglobulin E
ARIA	Allergic Rhinitis and its Impact on Asthma
ATP	Atopy patch test
AUC	Area under the curve
BAT	Basophil activation flow cytometry testing
BAT	Basophil activation test
BBEA	Peanut bead-based epitope assay
CAA	Current allergic asthma
CAR	Current allergic rhinitis
CBS	Consensus based statements
CD4+	Cluster of differentiation 4
CLIA '88	Clinical Laboratory Improvement Amendments of 1988
CLSI	Clinical and Laboratory Standards Institute
CMS	Centers for Medicare & Medicaid Services
CRS	Chronic rhinosinusitis
CW	Choosing wisely
EAACI	European Academy of Allergy and Clinical Immunology
EDTA	Ethylenediaminetetraacetic Acid
EP	Expert panel
FA	Food allergy
FcεRI	High-affinity IgE receptor
FDA	The Food and Drug Administration
FEV1	Forced expiratory volume in 1 second
GA2LEN	Global Allergy and Asthma European Network

H1	H-1 receptor antagonists
H2	H-2 receptor antagonists
IgA	Immunoglobulin A
IgD	Immunoglobulin D
IgE	Immunoglobulin E
IgE-FAB	Immunoglobulin E- fragment antigen-binding region
IgG	Immunoglobulin G
IgG4	Immunoglobulin A
IgM	Immunoglobulin A
JTFPP	Joint Task Force on Practice Parameters
LCDs	Local coverage determinations
LDTs	Laboratory-developed tests
LEAP	Learning early about peanut allergy
MBB	Mucosal brush biopsies
MFI	Median fluorescence intensity
NASEM	The National Academies of Science, Engineering and Medicine
NIAID	National Institute of Allergy and Infectious Diseases
NICE	National Institute for Health and Care Excellence
NPV	Negative predictive value
NSAIDs	Nonsteroidal anti-inflammatory drugs
OFC	Oral food challenge
PAMD@	Precision allergy molecular diagnostic applications
PPV	Positive predictive value
RARS	Recurrent acute rhinosinusitis
SAR	Seasonal allergic rhinitis
sIgE	Specific immunoglobulin E
s-IgE	Specific immunoglobulin E
SPT	Skin prick tests
SSRIs	Selective serotonin reuptake inhibitors
ST	Skin test
Th2	T helper type 2
tIgE	Total immunoglobulin E
WAO	World Allergy Organization

Scientific Background

Allergies affect over 50 million Americans, including approximately 30 percent of adults and 40 percent of children (Jackson et al., 2013; NASEM, 2016). The incidence of allergic disease is increasing (Pawankar et al., 2013) and is estimated to result in over $17 billion in health care costs and 200,000 emergency department visits annually (Adams et al., 2013).

A majority of environmental, food, and medication allergies with clinical significance are type I immunoglobulin E (IgE)-mediated allergies (Kowal & DuBuske, 2022). Diagnosis of an IgE-mediated allergy involves identification of the allergen, demonstration of IgE specific to that allergen, and confirmation that symptoms occur when the patient is exposed to the allergen. The IgE response to an

allergen can be assessed using skin or serum testing. Patch testing is preferred for delayed T-cell mediated response (Chang & Guarderas, 2018; Zug et al., 2014).

Allergic diseases, respiratory infections, and autoimmune conditions have similar clinical presentations and self-reported symptoms have a relatively low positive predictive value (PPV) (Sampson et al., 2014). Thus, laboratory allergy and immunologic testing are useful in clarifying diagnosis and guiding treatment when the frequency, duration, and sequelae of upper respiratory infections exceed the norm or when rhinosinusitis or asthma symptoms persist despite treatment (Chow et al., 2012). Allergy testing is also useful in identifying causative allergen in atopic dermatitis (eczema), contact dermatitis, urticaria, angioedema, and food or drug allergies. Knowing the causal allergen helps provide clinically relevant information for avoidance and treatment (Chang & Guarderas, 2018).

Skin Testing

Skin testing is the most rapid, sensitive, and cost-effective testing modality for the detection of immunoglobulin E (IgE)-mediated disease. The procedure lasts less than an hour with minimal patient discomfort. There are several published practice parameters for allergen skin testing (Bernstein et al., 2008; Chang & Guarderas, 2018; Kowal & DuBuske, 2022).

Serum IgE

Immunoglobulin E (IgE) is one of five immunoglobulins and the one primarily involved in allergic disease. At the cellular level, the allergic response starts with "atopy," a genetic predisposition to produce specific IgE after exposure to allergens. CD4+ helper T cells are predisposed to the "T helper type 2" (Th2) response, which causes the Th2 cells to secrete large amounts of interleukins 4 and 13, which then promotes production of the allergen specific IgE. From there, the allergen-specific IgE binds to high-affinity receptors on mast cells and basophils. At this point, if the relevant allergen is ingested in large enough amounts, the IgE molecules may cluster (cross-linking). This cross-linking causes the mast cells and basophils to release chemical and protein mediators, resulting in the characteristic allergic response (Stokes & Casale, 2022).

Immunoassays measuring both total IgE and allergen specific IgE in serum and other bodily fluids have been developed. Specific IgE immunoassays do not require patient cooperation, are not limited in patients with skin disease, are not blocked by antihistamines, and pose no risk of adverse reactions (Bernstein et al., 2008; Chang & Guarderas, 2018; Kowal & DuBuske, 2021; Stokes & Casale, 2022). Total IgE is usually unrelated to IgE levels for a specific allergen but may be useful in other conditions, such as asthma (Stokes & Casale, 2022).

Other testing

Patch testing is the gold standard for identification of a contact allergen (Mowad, 2006; Rietschel, 1997). Although occlusive patch testing is the most common technique, open, prophetic (provocative), repeated insult, photopatch, and atopy patch tests are also available if special situations indicate their use (Bernstein et al., 2008).

Cellular activation assays measuring the release of histamine from basophils (Kim et al., 2016; Santos & Lack, 2016) or mast cells (Bahri et al., 2018) as diagnostic or prognostic indicators of allergy have been the subject of intense research. Basophil and eosinophilic reactivity tests have been found to be associated with food-induced allergic responses and have been shown in current research to be modified over time during immunotherapy (Sampson et al., 2014). In particular, the basophil activation test (BAT) has

emerged as having superior specificity and comparable sensitivity to diagnose food allergies when compared with skin prick test and specific IgE (Santos & Shreffler, 2017). Histamine release from leukocytes of allergic persons is an excellent in vitro correlate of allergy; however, it is currently still considered a research test by the Academy of Allergy, Asthma, and Immunology (AAAI) (Bernstein et al., 2008).

Basophil activation flow cytometry testing (BAT) has the potential to be a useful tool for measuring hypersensitivity to allergens, especially for patients who are not suitable for skin testing due to skin status or prior severe reactions since it is an ex vivo, flow cytometry-based assay. BAT, for use as standard clinical practice, is currently limited by its lack of standardization in methodology as well as between systems used. A study by Depince-Berger et al. (2017) has proposed standardization between systems and instruments using whole blood-ethylenediaminetetraacetic acid (EDTA) samples with instrumentation standardization. "BAT would strongly benefit from easy implementation [EDTA, one step stimulation/labeling, wash, full sample analysis over time parameter, B cell relative basophil count] and standardization of instrument settings on MFI [median fluorescence intensity] targets whatever system or instrument is used" (Depince-Berger et al., 2017). Hemmings et al. (2018) note that standardization, quality assurance, and clinical validation will facilitate the transition of the BAT from research to clinical practice.

Proprietary testing

The Antigen Leukocyte Antibody Test (ALCAT) is another test available for the assessment of allergens. ALCAT measures food/immune reactions through stimulation of leukocytes. The immunological reactions to this stimulation are intended to identify sensitivities regardless of pathway as antibodies do not necessarily need to be involved. Cell Science Systems suggests individuals with a variety of disorders (such as gastrointestinal, neurological, et al.) to take this test (Cell Science Systems, 2023). Although the ALCAT machine is FDA registered and there are a few papers published, results are not reproducible when subject to rigorous testing and do not correlate with clinical evidence of allergy (Beyer & Teuber, 2005; Hammond & Lieberman, 2018; Wuthrich, 2005).

Panels encompassing a large number of analytes are also offered by labs. For example, Genova Diagnostics offers a blood test for IgG and IgE antibodies for 87 different foods. Genova also offers several variations on this test, such as "Vegetarian" (21 foods), "Spices" (23 spices), "Molds" (15 molds), and more (Genova, 2023).

Spiriplex offers a microarray-style panel for allergen testing, called "Allergenex." This test contains many purified allergen proteins to which a patient's blood sample can bind. This binding creates a quantifiable signal that allows the user to identify the number of IgE antibodies present, and therefore provide a picture of allergy. Spiriplex offers a test for 28 common food allergens, a test for 40 inhalant allergens, and 68 combined food and inhalant allergens (Spiriplex, 2023).

The VeriMAP Peanut Dx and the VeriMAP™ Peanut Sensitivity are both peanut-allergen specific bead-based epitope assays manufactured by AllerGenis LLC. According to Allergenis, VeriMAP™ has a "95% positive predictive value and can reduce overdiagnosis and anxiety by minimizing false positives" (BioSpace, 2021). This is an emerging technology and additional peer-reviewed literature establishing the analytical validity, clinical validity, and clinical utility of such testing will be further required.

Analytical Validity

Variables that can influence the wheal size when performing skin prick tests (SPT) include multiple operators, extract concentrations and quality, skin test devices, time of day, location on the skin, and the measuring of results (Nelson, 2001; Werther et al., 2012).

In 2006, Oppenheimer and Nelson evaluated variability and analytical validity of skin testing. A questionnaire was sent to all physician and fellow members of the American College of Allergy, Asthma and Immunology who were currently practicing in the United States. The objective of this questionnaire was to determine the diversity of skin testing practices among allergists. The results showed great variability among physicians. In particular, "The average number of skin prick tests performed ranged from 5.09 (grasses) to 10.9 (trees), whereas the average number of intradermal tests performed ranged from 2.03 (grasses) to 5.6 (perennial). The allergen extract concentrations used for intradermal testing varied widely. Expressed as a dilution of the concentrated extracts, 20.8% use 1:100 dilutions, 10.3% use 1:500 dilutions, and 59.4% use 1:1,000 dilutions. Significant variability also occurred regarding devices and the technique with which the devices were used. Most clinicians (92.1%) used the most concentrated extract available for skin prick testing. For reporting the results of skin testing, 53.8% used a 0 to 4+ scale, and only 28.3% measured orthogonal diameters. Of those using a 0 to 4+ scale, two thirds related the results to the size of the histamine control" (Oppenheimer & Nelson, 2006). The results from this survey emphasize potential areas of improvement for allergists regarding skin test use and data.

The Clinical and Laboratory Standards Institute (CLSI) has evaluated the analytical validity of serum IgE measurements and found that "Clinical/diagnostic sensitivity and specificity of IgE antibody assays cannot be accurately determined due to the absence of definitive gold standard methods for defining allergic disease. Total and allergen-specific IgE analyses achieve among the highest analytical performance of any antibody assay by following consensus procedures in CLSI-ILA20-A3" (Hamilton et al., 2015).

Knight et al. (2018) "examined the qualitative concordance between SPT and sIgE as measured on the HYTEC™288 platform for 10 commonly encountered inhalant allergens"; a total of 232 subjects were included. Overall concordance between SPT and sIgE was >70% for all allergens tested. Sensitivity ranged from 25% to 95% depending on the allergen, while specificity was significantly higher for all allergens (78-97%). Negative predictive value (NPV) was >85% for all allergens tested, while PPV was more variable, ranging from 22% to 88%. The authors noted that "these results are similar to findings in other studies comparing SPT with sIgE" (Knight et al., 2018).

Carlsson et al. (2015) examined the inter- and intra- variability of IgE and IgE receptor expression in the blood of seasonal allergic rhinitis (SAR) subjects. Thirty-two patients with SAR were included; the high-affinity IgE receptor, also known as FcεRI, and the low affinity receptor, also known as CD23, were measured. The authors found that "FcεRI expression on basophils and CD23 expression on B cells showed low intrasubject variability both in and out of the pollen season," although there was a small seasonal difference with lower total IgE levels and FcεRI expression during the pollen season (Carlsson et al., 2015).

Siroux et al. (2017) explored the effect of allergen nature, route of exposure, and dose of exposure on IgE and IgG responses. A total of 340 patients (170 with asthma, 170 without) were included, and IgE/IgG responses to 47 inhalant and food allergens were analyzed and compared between five French regions according to route of allergen exposure (inhaled or food). "Ubiquitous" allergens (grass, olive/ash pollen, house dust mites) did not show marked difference in specific IgE level between regions. For region-specific allergens (ragweed, birch, cypress), IgE sensitization was associated with regional pollen exposure. Airborne allergens cross-reacting with food allergens led to frequent IgG recognition. The authors concluded that "the variability in allergen-specific IgE and IgG frequencies depends on exposure, route of

exposure, and overall immunogenicity of the allergen. Allergen contact by the oral route might preferentially induce IgG responses" (Siroux et al., 2017).

Sookrung et al. (2019) measured the agreement of a SPT and serum specific IgE test to Periplaneta americana (American cockroach, ACR) allergies. ACR-extract was used, and sera was obtained from 66 individuals clinically diagnosed with chronic allergic rhinitis. Of the 66 samples, 46 were positive and 20 negative after a SPT to ACR-extract. Serum IgE levels were then measured by a commercial test kit. The authors note that of the SPT positive cases to ACR-extract, only 32.6% were also positive for serum IgE, indicating low concordance between the two testing methods (Sookrung et al., 2019).

He and Reisacher (2019) measured the sensitivity, specificity, and predictive value of oral mucosal brush biopsies (MBB) as a new diagnostic test for peanut allergies. Twenty individuals participated in this study; each participant underwent oral MBB and serum testing for peanut IgE. The authors note that "At 0.12 kU/L, the sensitivity of oral MBB testing was 80% and the specificity was 85%, whereas at 1.0 kU/L, the sensitivity of sIgE testing was 50% and the specificity was 100%. From the ROC curves, the areas under the ROC curve (AUC) for oral MBB and sIgE were 0.91 ($p < 0.001$) and 0.74 ($p = 0.007$), respectively. Combination testing further increased both sensitivity and accuracy over oral MBB alone" (He & Reisacher, 2019). These results are promising for oral MBB, although more research needs to be completed.

Clinical Utility and Validity

In 1998, Tschopp et al. (1998) compared three diagnostic tests for atopic diseases. Total serum IgE, Phadiatop, and the SPT were compared for 8329 individuals. Current allergic asthma (CAA) and current allergic rhinitis (CAR) were the conditions studied. The prevalence of CAA was 1.8% and prevalence for CAR was 16.3%. The prevalence of positive tests was 29%, 23%, and 23% for Phadiatop, SPT, and IgE, respectively. The results were as follows: "To diagnose current allergic asthma (CAA) and current allergic rhinitis (CAR), the sensitivity of Phadiatop was significantly higher than that of SPT (72.5% vs 65.4%, 77.1% vs 68.4% respectively) and IgE (72.5% vs 56.9%, 77.1% vs 43.9%, respectively. The sensitivity of SPT was significantly higher (68.4% vs 43.9%) than that of IgE to diagnose CAR. When CAA and CAR were excluded, the SPT specificity was significantly higher than that of Phadiatop (77.8% vs 71.9% and 85.9% vs 80.5%, respectively): when CAR was excluded, SPT was significantly higher than IgE (85.9 vs 81.4%). SPT had significantly the best positive predictive value for CAA (5.2% for SPT vs 4.6% for both IgE and Phadiatop) and CAR (48.7% for SPT vs 43.5% for Phadiatop and 31.6% for IgE). The three markers of atopy had roughly the same negative predictive value (NPV) for CAA, but IgE had a significantly lower NPV for CAR than SPT and Phadiatop (88.1% vs 93.3% and 94.7%, respectively). The diagnostic efficiency of SPT was significantly higher than that of Phadiatop (83.1% vs 79.9% and 77.6 vs 71.9%, respectively) to diagnose CAR and CAA. IgE and SPT had equal efficiency (77.6%), which was significantly higher than that of Phadiatop, to diagnose CAA (71.9%)" (Tschopp et al., 1998). The authors concluded that "SPT have the best positive predictive value and the best efficiency to diagnose respiratory atopic diseases. Furthermore, SPT give information on sensitivity to individual allergens and should therefore be used primarily by clinicians to assess respiratory allergic diseases" (Tschopp et al., 1998).

A retrospective analysis included patients who had been prick tested to "establish whether an incomplete diagnosis would have been reached if patch testing had been omitted." The authors observed that if "investigation of allergic skin disease is undertaken by a non-dermatologist, it is unlikely that patch testing will be performed." A total of 330 patients had been prick tested in the time period specified. Sixty-eight patients had positive reactions on prick testing, and 36 of those had positive patch tests. Of the 262 patients who had negative prick tests, 121 had positive patch tests (46.1%) of current relevance to patient

history in 92 subjects (35.1%). The authors concluded that "omission of patch testing from the investigation of allergic skin disease, even when contact urticaria may be the sole suspected diagnosis, would result in the frequent missed diagnosis of contact allergy" (Usmani & Wilkinson, 2007).

In 2014, a meta-analysis examined the clinical validity of SPT and IgE measurement for food allergy. Twenty-four studies consisting of 2831 participants were included. The results were as follows: "For cows' milk allergy, the pooled sensitivities were 88% (SPT), and 87% (IgE) and specificities were 68% and 48%. For egg, pooled sensitivities were 92% and 93% and specificities were 58% and 49% for SPT and specific-IgE. For wheat, pooled sensitivities were 73% and 83% and specificities were 73% and 43% for SPT and sIgE. For soy, pooled sensitivities were 55% and 83% and specificities were 68% and 38% for SPT and sIgE. For peanut, pooled sensitivities were 95% and 96%, and specificities were 61% and 59% for SPT and sIgE" (Soares-Weiser et al., 2014).

Klemans et al. (2015) examined the diagnostic accuracy of using sIgE to peanut components to improve sensitivity and specificity of peanut allergen testing. Twenty-two studies were included. The authors found that "sIgE to Ara h 2 [a peanut component] showed the best diagnostic accuracy of all diagnostic tests to diagnose peanut allergy. Compared to the currently used SPT and sIgE to peanut extract, sIgE to Ara h 2 was superior in diagnosing peanut allergy" (Klemans et al., 2015). The authors also found that the worst accuracy was observed to be sIgE to Ara8 and Ara9. The authors concluded that "sIgE to Ara 2 should replace SPT and sIgE to peanut extract in daily clinical practice" (Klemans et al., 2015).

Caglayan Sozmen et al. (2015) examined the diagnostic accuracy of using the patch test to avoid oral food challenge (OFC). They found that in 243 children that underwent OFC to suspected food, clinically relevant food allergies were seen in 40 (65%) children to egg and in 22 (35%) to cow's milk. The sensitivity of SPT for both milk and egg were 92%, specificity 91%, PPV 35%, and NPV 93%. Sensitivity, specificity, PPV, and NPV of atopy patch test for both milk and egg were 21%, 73%, 20%, and 74%, respectively.

Santos et al. (2014) studied the performance of basophil activation tests (BAT) as a diagnostic marker for peanut allergy. Forty-three peanut-allergic children, 36 peanut-sensitized but tolerant children, and 25 non–peanut-sensitized nonallergic children underwent SPT, sIgE, and BAT. The authors found that BAT in peanut-allergic children showed a peanut dose-dependent upregulation of CD63 and CD203c while there was no significant response in the other two cohorts. BAT optimal diagnostic cutoffs showed 97% accuracy, 95% PPV, and 98% NPV. BAT allowed reduction of required oral food challenges (OFCs) by two-thirds. BAT proved particularly useful in cases in which specialists could not accurately diagnose peanut allergy with SPT and sIgE to peanut and to Arah2. Using a two-step diagnostic approach in which BAT was performed only after equivocal SPT or Arah2-sIgE, BAT had a major effect (97% reduction) on the number of OFCs required.

Santos et al. (2015) also studied the utility of BAT to predict the severity and reactivity to peanut during OFCs. They found that "Of the 124 children submitted to OFCs to peanut, 52 reacted with clinical symptoms that ranged from mild oral symptoms to anaphylaxis. Severe reactions occurred in 41% of cases, and 57% reacted to 0.1 g or less of peanut protein. The ratio of the percentage of CD63(+) basophils after stimulation with peanut and after stimulation with anti-IgE (CD63 peanut/anti-IgE) was independently associated with severity, whereas the basophil allergen threshold sensitivity CD-sens ($1/EC_{50} \times 100$, where EC_{50} = half maximal effective concentration) value was independently associated with the threshold of allergic reactions to peanut during OFCs. Patients with CD63 peanut/anti-IgE levels of 1.3 or greater had an increased risk of severe reactions (relative risk, 3.4). Patients with a CD-sens value of 84 or greater had an increased risk of reacting to 0.1 g or less of peanut protein (relative risk, 1.9)" (Santos et al., 2015). The authors concluded that "Basophil reactivity is associated with severity, and

basophil sensitivity is associated with the threshold of allergic reactions to peanut. CD63 peanut/anti-IgE and CD-sens values can be used to estimate the severity and threshold of allergic reactions during OFCs" (Santos et al., 2015).

Davila et al. (2015) explored the association between total IgE and severity of asthma. A total of 383 patients were included (129 mild, 82 moderate, and 172 severe). Serum IgE levels were noted to vary "markedly" (147% coefficient of variation). The authors did not find an association between total IgE and forced expiratory volume in one second (FEV1) or asthma severity; although, the severe subgroup had a higher percentage of patients with >400 IU/mL. Independent predictors of higher IgE were found to be younger age, sensitization to at least two allergens, male gender, and family history of asthma. The authors concluded that "we did not find a significant association between serum total IgE levels and asthma severity or airflow limitation, except for a higher percentage of patients with IgE > 400 IU/mL in the severe subgroup" (Davila et al., 2015).

Tannert et al. (2017) investigated the relevance of a positive skin test and positive IgE test to penicillin allergy. Twenty-five patients with positive results were given penicillin, and another 19 patients deemed allergic were included. However, only nine of the 25 patients given penicillin were challenge positive. Positive results from each test alone did not predict allergy. The authors concluded that "the best predictor for a clinically significant (IgE-mediated) penicillin allergy is a combination of a positive case history with simultaneous positive ST result and s-IgE or a positive challenge result" (Tannert et al., 2017).

Suárez-Fariñas et al. (2021) investigated the validity of the peanut BBEA diagnostic test on 133 subjects as well as on 82 additional subjects from another study, forming a cohort for a paper titled, "Accurate and reproducible diagnosis of peanut allergy using epitope mapping." The authors measured levels of IgE to epitopes evaluated against a threshold established prior to the study. The peanut BBEA diagnostic test diagnosed 93% of subjects accurately, with a sensitivity threshold of 92% and specificity of 94%. The positive predictive value (PPV) was 91%. The authors concluded that "the overall accuracy was found to be superior to existing diagnostic tests for peanut allergy including skin prick testing, peanut sIgE, and peanut component sIgE testing" (Suárez-Fariñas et al., 2021).

Guidelines and Recommendations

The American Academy of Allergy, Asthma, and Immunology (AAAAI) and the American College of Allergy, Asthma, and Immunology (ACAAI)

The AAAI and ACAAI published practice parameters in 2008 for allergy testing (Bernstein et al., 2008) which noted that "For individual patients, the choice of test allergens is guided by the history and physical examination and the physician's knowledge, training, and experience." The guidelines recommended that "Specific IgE immunoassays may be preferable to skin testing under special clinical conditions, such as widespread skin disease, patients receiving skin test suppressive therapy, uncooperative patients, or when the history suggests an unusually greater risk of anaphylaxis from skin testing." They also note that for both skin testing and in-vitro specific IgE testing, "the allergens selected … should be determined based on the patient's age, history, environment and living conditions (e.g., region of the country), occupation, and activities." Also, "The best indicators in the selection of appropriate pollens for clinical use are extensive prevalence in the air and concurrent allergy symptoms during annually recurrent seasons when such pollens are expected to be present in the ambient air."

The AAAAI and ACAAI guidelines also state, "As is the case with skin tests, a direct correlation cannot be assumed between the presence of specific IgE (sIgE) antibodies and clinical disease." Additionally, "sensitivity and the positive predictive value of both prick/puncture and specific IgE tests generally tend to be higher among pollens, stable anaphylactogenic foods, house dust mite, certain epidermals, and fungi compared with venoms, drugs, and chemicals."

With regards to total IgE testing, these groups indicate, "Measurements of total serum IgE concentration are of modest clinical value when used as a screen for allergic disease or for predicting the risk of allergic disease."

The AAAAI and ACAAI also note that "IgG and IgG subclass antibody tests for food allergy do not have clinical relevance, are not validated, lack sufficient quality control, and should not be performed."

Regarding basophil activation assays they state, "Histamine and leukotriene release measurements from human basophils after incubation with allergen are valuable research tools for in vitro investigations of allergy" (Bernstein et al., 2008).

Their practice parameter on drug allergy also states that "The basophil activation test is a recently described method of evaluating expression of CD63 on basophils after stimulation with an allergen. There are limited data using this method to evaluate patients with possible allergies to β-lactam antibiotics and nonsteroidal anti-inflammatory drugs (NSAIDs)" (Boyce et al., 2010).

They also recommend, "Because anaphylactic reactions cannot be distinguished from anaphylactoid, nonimmune occurrences, it has been recommended that plasma histamine, tryptase, and specific IgEs (if available) may be ordered at the time of reaction and skin tests be performed later" (Boyce et al., 2010).

In their 2014 practice parameter on food allergy (Sampson et al., 2014) they acknowledge: "Basophil and eosinophilic reactivity tests have been shown to be associated with food-induced allergic responses and have been shown in current research to be modified over time during immunotherapy."

Their 2014 practice parameter on rhinosinusitis also recommends to "Perform an evaluation for specific IgE antibodies to airborne allergens in patients with RARS or CRS." An updated practice parameter on rhinitis published in 2020 comments that local allergic rhinitis will often be associated with "negative skin prick tests (and intradermal tests, when performed) and absence of serum-specific IgE (sIgE) antibodies but a positive nasal allergen provocation test (NAPT) to aeroallergens (Dykewicz et al., 2020). With respect to vasomotor rhinitis, the authors state that "laboratory tests, skin prick tests, and sIgE are helpful only to exclude AR [allergic rhinitis]" (Dykewicz et al., 2020).

In this practice parameter, they also make the following summary concerning re-evaluation of food allergies in children and adolescents: "Summary Statement 11: Consider the natural course of allergies to specific foods when deciding on the frequency of food allergy follow-up evaluations, recognizing that allergies to certain foods (milk, egg, wheat, and soy) generally resolve more quickly in childhood than others (peanut, tree nuts, fish, and shellfish). These observations could support individualized follow-up (ie, roughly yearly re-evaluations of these allergies in childhood) with less frequent retesting if results remain particularly high (eg, >20-50 kUA/L). [Strength of recommendation: Moderate; C Evidence]" (Dykewicz et al., 2020).

In their 2015 practice parameter on anaphylaxis (Lieberman et al., 2015), they recommend "Skin tests and/or in vitro tests for specific IgE and challenge tests might be appropriate to help define the cause of the anaphylaxis."

They also recommend against routinely obtaining total serum IgE levels for the diagnosis of food allergy, however, because of the low PPV of self-reported symptoms and lack of pathognomonic signs on physical examination, they recommend that the accurate diagnosis of IgE-mediated food allergy should be aided by laboratory allergy testing, including skin prick and/or serum IgE testing. The clinician should use specific IgE tests (skin prick tests, serum tests, or both) to foods as diagnostic tools; however, testing should be focused on foods suspected of provoking the reaction, and test results alone should not be

considered diagnostic of food allergy. Moreover, "the diagnosis of food-induced anaphylaxis should be based on signs and symptoms in association with likely or known exposure to a food allergen", as "events mimicking anaphylaxis also can occur after the ingestion of food" (Lieberman et al., 2015).

In a Choosing Wisely (CW) report, the AAAAI recommends against performing "unproven diagnostic tests, such as immunoglobulin G (IgG) testing or an indiscriminate battery of immunoglobulin E (IgE) tests, in the evaluation of allergy" (AAAAI, 2012).

In another CW report, the AAAAI recommends against routine diagnostic testing in patients with chronic urticaria, stating that "skin or serum-specific IgE testing for inhalants or foods is not indicated, unless there is a clear history implicating an allergen as a provoking or perpetuating factor for urticaria" (AAAAI, 2012).

The AAAAI also published a 2020 practice parameter update on peanut allergy diagnosis. The authors recommend in favor of diagnostic skin prick test or sIgE testing for peanut allergy in patients with physician-judged high pretest probability of peanut allergy. Testing is also recommended prior to an oral food challenge for patients with moderate pretest probability of peanut allergy. Ara h 2 diagnostic testing is the suggested approach for patients presenting for evaluation of suspected peanut allergy for which a single diagnostic test is to be used, due to its superior diagnostic accuracy "by virtue of more optimal positive/negative likelihood ratios." However, Ara h 2 is noted to have lower sensitivity than the skin prick or sIgE tests, so a clinician may use Ara h 2, SPT, or sIgE to confirm the diagnosis of peanut allergy in a patient with a high prior probability. The AAAAI recommends against "routine use of component testing in addition to either SPT or sIgE to whole peanut to increase diagnostic accuracy," and against using the results of skin prick or sIgE to determine "the severity of an allergy phenotype or to predict the severity of a future reaction" (Greenhawt et al., 2020). It is noteworthy that all the recommendations above were assigned "low" or "very low" degrees of evidence certainty (Greenhawt et al., 2020).

Joint Task Force on Practice Parameters (JTFPP)

In a practice parameter concerning contact dermatitis, the Joint Task Force on Practice Parameters—composed of the American Academy of Allergy, Asthma & Immunology (AAAAI), the American College of Allergy, Asthma & Immunology (ACAAI), and the Joint Council of Allergy, Asthma & Immunology—proposed this series of summary statements:

"Summary Statement 1: Consider ACD [allergic contact dermatitis] in the differential diagnosis of patients with chronic eczematous or noneczematous dermatitis. [Strength of Recommendation: Strong; C Evidence]

Summary Statement 2: In patients suspected of ACD, patch testing is the gold standard to confirm the diagnosis. [Strength of Recommendation: Strong; C Evidence]

Summary Statement 3: In addition to personal products used by a patient suspected of ACD, review the home and workplace for other sources of contact allergens. [Strength of Recommendation: Moderate; D Evidence] Summary Statement

Summary Statement 4: Evaluate patients for both irritant and allergic causes, especially in those presenting with hand dermatitis. [Strength of Recommendation: Strong; C Evidence]

Summary Statement 5: Allergic CD should be suspected and evaluated in the patient with both generalized and anatomically localized skin eruptions (such as the hands, face, eyelids) that come in contact with the substances in the environment. [Strength of Recommendation: Moderate; C Evidence]

Summary Statement 6: In a patient with a facial rash involving the periorbital areas (eg, eyelids), evaluate for ACD caused by components of cosmetics, such as fragrances, preservatives, and excipients, because these are common sensitizers of the facial skin. [Strength of Recommendation: Moderate; C Evidence]

Summary Statement 7: Evaluate patients presenting with lip dermatitis (cheilitis) and perioral dermatitis for both irritant and allergic causes of contact dermatitis. [Strength of Recommendation: Moderate; C Evidence]

Summary Statement 8: Evaluate patients with chronic oral mucosal inflammatory conditions for disorders other than ACD. [Strength of Recommendation: Moderate; C Evidence]

Summary Statement 9: In patients presenting with dermatitis that involves the scalp and neck, consider patch testing for common causative sensitizers in cosmetics, hair products, and jewelry. [Strength of Recommendation: Moderate; C Evidence]

Summary Statement 10: Consider irritant and ACD in all patients presenting with acute or chronic hand eczema. All such patients suspected of CD should undergo patch testing. [Strength of Recommendation: Moderate; C Evidence]

Summary Statement 11: Evaluate patients with axillary dermatitis for ACD caused by local contact sensitivity to allergens in topically applied products found in deodorants and textiles. In some cases, axillary dermatitis could be a manifestation of systemic contact dermatitis (SCD) (i.e., "the baboon syndrome"). [Strength of Recommendation: Moderate; C Evidence]

Summary Statement 12: Evaluate patients presenting with anogenital dermatitis for possible ACD to antigens contained in topically applied products. [Strength of Recommendation: Moderate; C Evidence]

Summary Statement 13: Consider a diagnosis of SCD following systemic exposure (e.g., ingestion, infusion, or transcutaneous exposure) to a known contact sensitizer in a patient who presents with generalized dermatitis, intertriginous and flexural exanthema (Baboon syndrome), and/or a flare at previous cutaneous sites of exposure [Strength of Recommendation: Moderate; C Evidence].

Summary Statement 14: Consider PT to rubber chemicals, adhesives, and leather components of footwear in patients presenting with unexplained chronic dermatitis involving the lower extremities, feet and/or soles. [Strength of Recommendation: Moderate; C Evidence]

Summary Statement 15: In addition to avoiding irritants in patients with atopic dermatitis (AD), evaluate for ACD, if suspected, as the 2 dermatologic conditions often coexist in the same patient. [Strength of Recommendation: Moderate; C Evidence]" (Fonacier et al., 2015).

Consensus based statements (CBSs) regarding the diagnosis and management of rhinitis from the JTFPP include the following (Dykewicz et al., 2020):

Recommendation no.	CBS or GRADE recommendation	Strength of recommendation	Certainty of evidence
1	CBS: We recommend that the clinician complete a detailed history and a physical examination in a patient presenting with symptoms of rhinitis.	Strong	Low
2	CBS: We recommend that for patients presenting with rhinitis symptoms, a review of all current medications should be completed to assess whether drug-induced rhinitis may be present.	Strong	Ungraded
3	CBS: We recommend that aeroallergen skin prick testing or sIgE testing be completed to confirm the diagnosis of AR in a patient with a history consistent with AR.	Strong	High
4	CBS: We recommend that the clinician not perform food skin prick testing or sIgE for foods in their routine evaluation of a patient presenting with the signs and symptoms compatible with the diagnosis of AR.	Strong	Ungraded
5	CBS: We suggest that the use of a validated instrument (eg, scoring system, scale, or questionnaire) be considered to help determine the severity of rhinitis and to monitor the degree of disease control.	Conditional	Low

World Allergy Organization Position Paper

- In 2020, the World Allergy Organization published a position paper on IgE allergy diagnostics and other relevant allergy tests. Key statements from the paper can be found below:
- "Clinical suspicion of allergic sensitization is confirmed by demonstrating the presence of allergen-specific IgE antibodies *in vivo* (skin tests) or *in vitro*.
- Confirmation of allergen sensitization and the identification of causal allergens are essential for optimizing the management of allergic conditions.
- Skin prick testing (SPT) is the most frequently used method for the detection of IgE antibodies, due to its rapidity, simplicity, and low cost. Skin prick tests and other skin test results must be interpreted by a clinician with adequate knowledge of medical history, clinical findings, and relevant type I allergens (including environmental, food, animal, insect, fungal, and drug allergens). Skin tests should include the relevant allergens in the given geographical area and ideally carried out only using standardized allergenic extracts.
- *In vitro* tests, including molecular based allergy diagnostics, using either in single-plex and in multi-plexed strategies and other more functional tests, such as Basophil Activation Tests allow to better define the IgE profile of the patient. This approach is in line with the Precision Medicine statements" (Ansotegui, Melioli, Canonica, Caraballo, et al., 2020)

The paper also states that "Skin tests, especially SPT, represent the most reliable and cost-effective tool for the diagnosis and management of IgE-mediated diseases. They demonstrate a good correlation with outcomes of nasal, conjunctival, dermal, oral, and bronchial challenges" (Ansotegui, Melioli, Canonica, Caraballo, et al., 2020).

Clinical conditions where SPT is indicated include:

- "Asthma;
- Rhinitis/rhinosinusitis/rhino-conjunctivitis/conjunctivitis;
- Eczema/atopic dermatitis (in the setting of selectively high clinical suspicion for underlying presence of IgE hypersensitivity to specific allergens);
- Suspected food allergy (oral allergy syndrome, anaphylaxis/acute onset or exacerbation of urticaria or eczema that is temporally correlated with food ingestion);
- Suspected drug allergy;
- Hymenoptera venom allergy (systemic reactions immediately following insect sting);
- Suspected occupational disease or exposure to selected potential allergens;
- Chronic urticaria in rare selected cases which strongly suggest an allergen as potential trigger/ aggravating factor;

- Less common disorders, such as eosinophilic esophagitis, eosinophilic gastroenteritis, or allergic bronchopulmonary aspergillosis, where IgE sensitization is one of the characteristics of its pathogenesis. However, there is controversy regarding the utility of SPT for these illnesses" (Ansotegui, Melioli, Canonica, Caraballo, et al., 2020)

"SPT is not routinely indicated in the following instances in the absence of other existing features of allergic disease:

- Suspected food intolerance (e.g., irritable bowel syndrome, etc.);
- Chronic urticaria in the absence of allergic features in the history;
- Desire to lose weight (according to nonconventional approaches, obesity may be due to food intolerance, but no supporting scientific data have been reported in the literature);
- Non-specific food-associated symptoms to food additives/preservatives/colorants;
- Evaluation of the effectiveness of allergen immunotherapy (but may be supportive in Hymenoptera venom immunotherapy);
- Non-specific respiratory symptoms to irritants (i.e., smoke, perfumes, detergents, chemicals and other strong odors);
- Screening for allergic sensitization patterns in the absence of clinical symptoms (i.e., family history of allergy);
- Non-specific cutaneous rashes in the absence of atopic features or other allergic symptoms; migraine, except for the indication of specific hypersensitivity to hormones. However, strong scientific data are still missing.
- Chronic fatigue syndrome" (Ansotegui, Melioli, Canonica, Caraballo, et al., 2020)

In a 2020 publication on anaphylaxis guidance, the WAO confirms that "allergy testing should be based on patient history and local data regarding the common causes of anaphylaxis in the region. The most frequent elicitor groups worldwide are food, insect venom, and drugs" (Cardona et al., 2020).

World Allergy Organization (WAO), Allergic Rhinitis and its Impact on Asthma (ARIA), and the Global Allergy and Asthma European Network (GA^{2}LEN)

The WAO, ARIA, and GA2LEN published a consensus document in 2020 focused on molecular-based allergy diagnoses. Precision allergy molecular diagnostic applications (PAMD@) "can increase the accuracy of an allergy diagnosis in certain circumstances. In allergic patients, a molecular approach is suitable for the following:

- Assessing the risk of potential allergic reactions, which depend on the individual allergic (clinical) sensitization profile;
- Evaluating whether unknown potential triggering factors are present (i.e., the presence of sIgE versus allergenic molecules correlated with high risk for allergic reactions)" (Ansotegui, Melioli, Canonica, Gomez, et al., 2020).

National Institute of Allergy and Infectious Diseases (NIAID)

The NIAID convened an expert panel to review current information and to make recommendations related to the evaluation of food allergy (FA), including the use of specific IgE (sIgE) testing (Boyce et al., 2010). With regards to allergen-specific serum IgE determination, NIAID recommended that "sIgE tests for identifying foods that potentially provoke IgE-mediated food-induced allergic reactions, but alone these tests are not diagnostic of FA." It stated that "sIgE testing and skin prick testing both depend on the presence of allergen-specific antibodies. Because the former test measures sIgE in the serum and the latter reflects IgE bound to cutaneous mast cells, their results may not always correlate. Serum testing can be especially useful when SPTs cannot be done (for example, due to extensive dermatitis or dermatographism), or when antihistamines cannot be discontinued." The NIAID also

recommended not using the combination of skin prick test (SPT), sIgE tests and atopy patch test (ATP) for the routine diagnosis of food allergy.

Additionally, the NIAID notes that "the routine use of measuring total serum IgE should not be used to make a diagnosis of FA" (Boyce et al., 2010).

"Non-standardized tests" such as basophil histamine release/activation, lymphocyte stimulation, allergen-specific IgG, cytotoxicity assays, and mediator release assays should not be used in the routine evaluation of FA, according to the NIAID guidelines (Boyce et al., 2010).

In 2017, the NIAID published addendum guidelines for the prevention of peanut allergy in the United States. These guidelines note that the expert panel (EP) "recommends that evaluation with peanut-specific IgE (peanut sIgE) measurement, SPTs, or both be strongly considered before introduction of peanut to determine if peanut should be introduced and, if so, the preferred method of introduction. To minimize a delay in peanut introduction for children who may test negative, testing for peanut sIgE may be the preferred initial approach in certain health care settings, such as family medicine, pediatrics, or dermatology practices, in which skin prick testing is not routine" (Togias et al., 2017). Further, "The EP does not recommend food allergen panel testing or the addition of sIgE testing for foods other than peanut because of their poor positive predictive value, which could lead to misinterpretation, overdiagnosis of food allergy, and unnecessary dietary restrictions" (Togias et al., 2017). More, if an infant has severe eczema, an egg allergy, or both, the EP recommends to "Strongly consider evaluation by sIgE measurement and/or SPT and, if necessary, an OFC. Based on test results, introduce peanut-containing foods" (Togias et al., 2017).

American Academy of Pediatrics (AAP)

In 2012, AAP released a clinical report on allergy testing in childhood. It stated that "Both serum sIgE tests and SPT are sensitive and have similar diagnostic properties." The AAP summary included the following:

- "Treatment decisions for infants and children with allergy should be made on the basis of history and, when appropriate, identified through directed serum sIgE or SPT testing. Newer in vitro sIgE tests have supplanted radioallergosorbent tests."
- "Positive sIgE test results indicate sensitization but are not equivalent to clinical allergy. Large panels of indiscriminately performed screening tests may, therefore, provide misleading information."
- "Increasingly higher levels of sIgE (higher concentrations on serum tests or SPT wheal size) generally correlate with an increased risk of clinical allergy."
- "Use of a multiallergen serum test can be helpful for screening for atopic disease if there is a clinical suspicion. If positive, allergen-specific testing may be considered.
- "Tests for allergen-specific IgG antibodies are not helpful for diagnosing allergies" (AAP, 2012).

In 2019, the AAP published new guidelines on the prevention of childhood food allergies and other allergic conditions. This article states that "The new recommendations for the prevention of peanut allergy are based largely on the LEAP trial and are endorsed by the AAP." The AAP endorsed guidelines were published by Togias et al. (2017) and are noted above. They state that the highest-risk infants (those with severe eczema and/or egg allergies) should be introduced to peanuts by four to six months; further, allergy testing is strongly advised before peanut introduction. SPT and blood testing for peanut-specific IgE (sIgE) are allowable (Greer et al., 2019; Sicherer, 2017).

In 2020, the AAP published a state-of-the-art review of peanut allergy testing advances and controversies. The article states that "current first-line diagnostic tests for peanut allergy have limited specificity, which

may be enhanced with emerging tools such as component-resolved diagnostics." Like the 2019 guideline, they note that first-line best practices for peanut allergy testing include SPT or serum peanut-specific IgE measurement. While both tests are highly sensitive, neither correlate strongly with reaction severity, according to the AAP (Abrams et al., 2020).

U.S. Food and Drug Administration (FDA) on Xolair

The availability of Xolair for treatment of allergic asthma also has implications for allergy testing. According to the package insert, Xolair is indicated for patients six years of age and older with moderate to severe persistent asthma who have a positive skin test or in vitro reactivity to a perennial aeroallergen and whose symptoms are inadequately controlled with inhaled corticosteroids… Determine dose (mg) and dosing frequency by serum total IgE level (IU/mL) measured before the start of treatment, and by body weight (kg)." The prescribing information also notes that "Total IgE levels are elevated during treatment and remain elevated for up to one year after the discontinuation of treatment. Therefore, re-testing of IgE levels during Xolair treatment cannot be used as a guide for dose determination" (FDA, 2016).

International Consensus Statement on Allergy and Rhinology: Allergic Rhinitis

The authors reviewed the existing evidence behind various aspects of evaluation and diagnosis of the AR patient, and developed the following recommendations for AR diagnostic modalities (Wise et al., 2023):

- Patient history: "Using history to make a presumptive diagnosis of AR is reasonable and would not delay treatment initiation. History should be combined with physical examination, which may not be possible in some scenarios such as telemedicine. Confirmation with diagnostic testing is required for progression to AIT or targeted avoidance therapy, or desirable with inadequate response to treatment… Despite low level evidence specifically addressing this area, history is essential in the diagnosis of AR."
- Physical examination: "When possible, physical examination should be performed with appropriate personal protective equipment to aid in the diagnosis of AR and exclusion of other conditions. When combined with patient history, it increases diagnostic accuracy and may exclude alternative causes of symptoms."
- Nasal endoscopy: "Nasal endoscopy may be considered as a diagnostic adjunct in the evaluation of patients with suspected AR."
- Radiologic studies: "Routine use of imaging is not recommended for the diagnosis of AR."
- Use of validated subjective instruments and patient-reported outcome measures: "Validated surveys may be used to screen for AR, follow treatment outcomes and as a primary outcome measure for clinical trials. Specific tests are optimized for various clinicopathological scenarios."
- Skin prick testing: "Patients can benefit from identification of their specific sensitivities. Skin prick testing (SPT) is a quick and relatively comfortable way to test several antigens with accuracy similar to other available methods of testing… Regular use of the same SPT device type will allow clinicians to familiarize themselves with it and interpretation of results may therefore be more consistent. The use of standardized allergen extracts can further improve consistency of interpretation."
- Skin intradermal testing: "Intradermal skin tests may not perform as well as SPT in most clinical situations… Intradermal testing may be used to determine aeroallergen sensitization in individuals suspected of having AR."
- Blended skin testing techniques: "While AIT can be based off SPT results alone, endpoint-based immunotherapy may have possible benefits of decreased time to therapeutic dosage… Blended skin testing techniques, such as modified quantitative testing, are methods that can be used to determine a starting point for AIT or confirm allergic sensitization."
- Serum total immunoglobulin E: "Assessment of total IgE may be useful to assess overall atopic status; furthermore, in selected cases it might help guide therapy (i.e., predict outcome of AIT)."
- Serum allergen-specific immunoglobulin E: "Patients can benefit from identification of their specific sensitivities. Further, in some patients who cannot undergo SPT, serum sIgE testing is a safe and effective

alternative… Serum sIgE testing may be used in patients who cannot undergo allergy skin testing. The use of highly purified allergen or recombinants can increase the sensitivity, specificity, and diagnostic accuracy of sIgE tests. Rigorous proficiency testing on the part of laboratories may also improve accuracy."

- Nasal allergen-specific immunoglobulin E: "In patients with non-allergic rhinitis who also have risk factors for atopic disease and have inadequate response to pharmacotherapy, testing for nasal sIgE may be helpful in confirming a diagnosis of local AR and allowing for treatment with AIT. There is no consensus for levels of nasal sIgE that indicate sensitivity… Measurement of nasal sIgE is an option in patients with non-allergic rhinitis suspected of having local AR to support this diagnosis and guide AIT if pharmacologic therapies are inadequate. Consensus for levels of nasal sIgE indicating AR need to be established."
- Basophil activation test: "The evidence does not support routine use for the diagnosis of AR or for following AIT response… Application of basophil activation test in specific situations where other diagnostic procedures for AR are not possible or conflicting. Potentially useful for monitoring AIT if other methods fail or show conflicting results."
- Component resolved diagnostic testing: "Molecular diagnosis may be a useful tool for assessment of AR in some scenarios, especially in polysensitized patients… Component resolved diagnostic testing is an option for diagnosis of AR by specialists."
- Nasal provocation testing: "Application of nasal provocation testing is useful in local AR and to confirm occupational rhinitis."
- Nasal cytology: "Nasal cytology could help in cases of non-allergic rhinitis to suspect local AR or in cases of AR to diagnose a mixed rhinitis. It could be considered an option in cases of negative SPT and/or serum sIgE to evaluate the presence of mucosal eosinophils and consideration of local AR or type two inflammation. The cut-off values for determining non-allergic rhinitis with eosinophilia syndrome (NARES) are not yet clear."
- Nasal histology: "Nasal histology may be helpful in clinical research or selected cases (e.g., evaluation of tissue eosinophils during surgery). Recommendation against in routine clinical practice for AR evaluation due to invasive nature of obtaining a specimen."
- Rhinomanometry: "Rhinomanometry is useful in distinguishing between structural and soft tissue causes of obstruction, when history and examination findings are not congruent, as well as a research tool. Better with individual nasal cavity assessment and four-phase rhinomanometry."
- Acoustic rhinometry: "Acoustic rhinometry is most useful in research setting as opposed to as a clinical diagnostic tool."
- Peak nasal inspiratory flow: "Use in conjunction with patient reported outcome measures to improve utility."
- Nitric oxide measurements: "There is inconsistent evidence in the ability of FeNO or nNO to differentiate adults and children with AR and non-allergic rhinitis. Most studies were of low evidence or small impact. There is no agreed upon cut-off value when performing FeNO or nNO for the diagnosis of AR… History and physical, diagnostic skin testing, or sIgE testing should be the first-line evaluation of AR. FeNO or nasal NO testing may provide additional diagnostic information if necessary but should not be routinely employed for AR diagnosis."

The National Academies of Science, Engineering and Medicine

The National Academies of Science, Engineering and Medicine convened an expert committee to review the science and management practices of food allergy. Overall, they found that:

- "Currently, no simple diagnostic tests exist for food allergy."
- "Food allergy evaluation procedures include a medical history and physical examination, and also may include food-specific skin prick test, food-specific serum immunoglobulin E test, diagnostic food elimination diet, and oral food challenge (OFC). Selection of the specific tests needs to be individualized based on the medical history of each patient."

- "The BAT shows promising preliminary data, the potential utility is recognized and will require additional validation and standardization. "Guidelines suggest not using the BAT clinically on the grounds that it is nonstandardized, but recognize its use as a research tool" (NASEM, 2016).

In 2017, the National Academies of Science, Engineering and Medicine convened an expert committee to examine critical issues related to food allergy. Regarding diagnosis and prognosis, the committee notes that "physicians [should] use evidence-based, standardized procedures as the basis for food allergy diagnosis and avoid nonstandardized and unproven procedures…. When food allergy is suspected, the patient should be evaluated by a physician who has the training and experience to select and interpret appropriate diagnostic tests" (Sicherer et al., 2017).

American Academy of Family Physicians (AAFP)

American Academy of Family Physicians recommendations for practice state: "Allergy and immunologic testing can help clarify the diagnosis and guide treatment. Immediate immunoglobulin E (IgE) and delayed T cell–mediated reactions are the main types of allergic responses. The allergens suspected in an immediate IgE-mediated response are identified through serum IgE-specific antibody or skin testing. For patients with an inhalant allergy, skin or IgE-specific antibody testing is preferred. In patients with food allergies, eliminating the suspected allergenic food from the diet is the initial treatment. If this is ineffective, IgE-specific antibody or skin testing can exclude allergens. An oral food challenge should be performed to confirm the diagnosis. Patients with an anaphylactic reaction to an insect sting should undergo IgE-specific antibody or skin testing. Skin testing for penicillin has a high negative predictive value and can help when penicillin administration is indicated and there are limited alternatives. Testing for other drug allergies has less well-determined sensitivity and specificity but can guide the diagnosis. Patch testing can help identify the allergen responsible for contact dermatitis" (Chang & Guarderas, 2018).

European Academy of Allergy and Clinical Immunology (EAACI)

The EAACI published guidelines on "Biomarkers for monitoring the clinical efficacy of allergen Immunotherapy (AIT)." In it, they concluded that "to date, there are no validated and generally accepted candidate biomarkers that are predictive or indicative of the clinical response to AIT." However, they did note sIgE/tIgE ratio and IgE-FAB as candidate biomarkers for future research (Shamji et al., 2017).

The EAACI released a position statement on the BAT. In it, they concluded that "Basophil activation test has been established as a routine diagnostic test with standardized allergen preparations in a number of service laboratories… An important next step is the standardization and automation of analysis of BAT. Once that is achieved, it will be possible to do large multicenter trials to characterize the diagnostic performance of BAT and broaden its use as a clinical tool" (Hoffmann et al., 2015).

The EAACI released a Molecular Allergology User's Guide 2.0. While not a formal guideline, the guide provides comprehensive information about molecular allergen testing. In terms of IgE antibody testing, guide notes that "The clinical relevance of allergen-specific IgE detection in a patient's serum is strictly as a marker for allergic sensitisation (risk for allergy) and it alone cannot predict the probability of an allergic reaction. The determination of the diagnostic sensitivity and specificity of IgE antibody assays will thus remain difficult to definitively determine because of the lack of an absolute (gold standard) method of defining the presence of allergic disease. This means that the clinical relevance of an allergic sensitisation (i.e. presence of allergen-specific IgE) independent of the use of allergen extracts or molecules for diagnostic purposes will ultimately be determined only by the physician and not by the test" (Dramburg et al., 2023).

In terms of basophil activation testing, the guide notes that "The BAT can be useful to confirm the diagnosis of food, venom and respiratory allergies" (Dramburg et al., 2023).

In terms of *In vivo* testing, the guide notes that "provocation tests are especially helpful when discrepancies exist between the clinical history and other in vivo or in vitro test results, to phenotype patients and to monitor the

efficacy of allergen-specific immunotherapy" but further states that "the use of recombinant allergens in provocation tests seems to improve their accuracy; however, it is an unmet need which requires further investigations" (Dramburg et al., 2023).

National Institute for Health and Care Excellence (NICE)

National Institute for Health and Care Excellence published a guideline on asthma, recommending against use of serum total or specific IgE for diagnosing asthma. Specific IgE or prick tests to aeroallergens should be used to identify triggers to asthma after a formal diagnosis has been made (NICE, 2021).

The NICE also released a statement on multiplex allergen testing, particularly "ImmunoCAP ISAC" Although they acknowledge the test's promise, they state that there is "insufficient evidence to recommend the routine adoption of multiplex allergen testing with ImmunoCAP ISAC 112 to help diagnose allergy and predict the risk of an allergic reaction in people with allergy that is difficult to diagnose, when used with standard clinical assessment" (NICE, 2020).

Regarding the assessment and diagnosis of food allergy in under 19s, NICE published the below recommendations:

For food allergies classified as IgE-mediated:

"Based on the results of the allergy-focused clinical history, if IgE-mediated allergy is suspected, offer the child or young person a skin prick test and/or blood tests for specific IgE antibodies to the suspected foods and likely co-allergens."
"Tests should only be undertaken by healthcare professionals with the appropriate competencies to select, perform and interpret them."
"Skin prick tests should only be undertaken where there are facilities to deal with an anaphylactic reaction."
"Choose between a skin prick test and a specific IgE antibody blood test based on:

- the results of the allergy-focused clinical history **and**
- whether the test is suitable for, safe for and acceptable to the child or young person (or their parent or carer) **and**
- the available competencies of the healthcare professional to undertake the test and interpret the results."

"Do not carry out allergy testing without first taking an allergy-focused clinical history. Interpret the results of tests in the context of information from the allergy-focused clinical history."
"Do not use atopy patch testing or oral food challenges to diagnose IgE-mediated food allergy in primary care or community settings" (NICE, 2011).

Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: http://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved

or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
82784	Gammaglobulin (immunoglobulin); IgA, IgD, IgG, IgM, each
82785	Gammaglobulin (immunoglobulin); IgE
82787	Gammaglobulin (immunoglobulin); immunoglobulin subclasses (eg, IgG1, 2, 3, or 4), each
83516	Immunoassay for analyte other than infectious agent antibody or infectious agent antigen; qualitative or semiquantitative, multiple step method
86001	Allergen specific IgG quantitative or semiquantitative, each allergen
86003	Allergen specific IgE; quantitative or semiquantitative, crude allergen extract, each
86005	Allergen specific IgE; qualitative, multiallergen screen (eg, disk, sponge, card)
86008	Allergen specific IgE; quantitative or semiquantitative, recombinant or purified component, each
88184	Flow cytometry, cell surface, cytoplasmic, or nuclear marker, technical component only; first marker
88185	Flow cytometry, cell surface, cytoplasmic, or nuclear marker, technical component only; each additional marker
0165U	Peanut allergen-specific quantitative assessment of multiple epitopes using enzyme-linked immunosorbent assay (ELISA), blood, individual epitope results and probability of peanut allergy Proprietary test: VeriMAP™ Peanut Dx – Bead-based Epitope Assay Lab/Manufacturer: AllerGenis™ Clinical Laboratory
0178U	Peanut allergen-specific quantitative assessment of multiple epitopes using enzyme-linked immunosorbent assay (ELISA), blood, report of minimum eliciting exposure for a clinical reaction Proprietary test: VeriMAP™ Peanut Sensitivity - Bead Based Epitope Assay Lab/Manufacturer: AllerGenis™ Clinical Laboratory

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

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Revision History

Revision Date	Summary of Changes
03/06/2024	Reviewed and Updated: Updated the background, guidelines and recommendations, and evidence-based scientific references. Literature review did not necessitate any modifications to coverage criteria. The following edits were made for clarity and consistency: CC3 edited for clarity and consistency to allow enhanced enforcement. CC7 and CC8 were wrapped into a single DNMCC criterion for individuals with signs and symptoms of allergies. Now reads: "7) For individuals with signs or symptoms of allergies, basophil activation flow cytometry testing (BAT) and in-vitro testing of IgG, IgA, IgM, and/or IgD DO NOT MEET COVERAGE CRITERIA."
03/01/2023	Reviewed and Updated: Updated the background, guidelines and recommendations, and evidence-based scientific references. Literature review did not necessitate any modifications to coverage criteria. The following edits were made for clarity: All CC edited for clarity and consistency. CC1b broken into two subcriteria for clarity. Previously read "b) When skin testing is either contraindicated (see Policy Guidelines below for details), or when direct skin testing results are not consistent with the history of an anaphylactic or other severe reaction to an allergen and further treatment decisions would be impacted by confirmation of sensitivity, in the evaluation of: i) individuals with asthma, or ii) individuals with suspected allergen-induced chronic rhinitis, or

	iii) individuals with suspected food allergy, or iv) individuals with suspected insect venom allergy, or v) individuals with suspected allergy to specific drugs" Now reads: "b) When skin testing is contraindicated (see Note 1). c) When further treatment decisions would be impacted by confirmation of sensitivity in individuals for whom direct skin testing results are not consistent with the history of an anaphylactic or other severe reaction to an allergen." CC2 changed from subcriteria to a single main criteria, now reads: "2) When limited to allergens chosen for testing based on an individual's history, physical examination, and environment, specific IgE in-vitro allergy testing (up to 20 allergen specific antibodies per year) MEETS COVERAGE CRITERIA."
03/09/2022	Off cycle review: Updated background, guidelines and recommendations, and evidence-based scientific references. Literature review necessitated the addition of a new CC4 that reads "In individuals with positive food allergen results, annual re-testing for the same food allergens MEETS COVERAGE CRITERIA in children and adolescents to monitor for allergy resolution." Edited CC5 for clarity with the addition of new CC "Except as specified in criteria 4 (above), routine re-testing for allergies to the same allergens DOES NOT MEET COVERAGE CRITERIA in the absence of a new clinical presentation." Removed "When in-vitro testing is ordered, the medical record must clearly document the indication and why it is being used instead of skin testing." from CC1a. Now reads: "a) In lieu of skin testing for an INITIAL allergy screen." Removed CPT code 83520
09/01/2021	Annual review: Updated background, guidelines, federal/state regulation section, and evidence-based scientific references. Literature review did necessitate change in coverage criteria: Addition for clarity of CC9: • In-vitro testing of allergen non-specific IgE that does not identify a specific allergen using qualitative multi-allergen screen DOES NOT MEET COVERAGE CRITERIA in the evaluation of suspected allergy and for any other indication.
09/08/2020	Annual review: Updated background, guidelines, and evidence-based scientific references. Switched the E&I CC to DNMCC with the preceding statement regarding lack of published scientific literature. New CC added: "In-vitro allergen testing using bead-based epitope assays such as VeriMAP Peanut Dx and others **DOES NOT MEET COVERAGE CRITERIA**". CPT 88185 and PLAs 0165U and 0178U were added.
10/04/2019	Annual review: Updated background, guidelines and recommendations, federal regulations, and evidence-based scientific references. The following modifications have been made to the coverage criteria: • Removed "Additional testing beyond this number will require individual review for coverage criteria" from CC2 because of the inability of enforcement and to decrease confusion within the CC. • Added "or non-specific IgG, IgA, IgM, and/or IgD" to the CC regarding IgG testing as **E&I** to aid in enforcement due to multiple possible CPT codes.

	Removed: 88346, 86352, 86021, 86343
9/25/2018	Annual review: Description, Literature Review, Federal Regulations, Guidelines and Recommendations, and Scientific References were updated. Added CC6 per NIAID, 2010& NASEM, 2016: "Basophil Activation flow cytometry testing (BAT) for measuring hypersensitivity to allergens is considered **EXPERIMENTAL AND INVESTIGATIONAL."** CPT 82784 was changed from PA required to Not Covered.
1/1/2018	Off cycle review: added new 2018 CPT code of 86008 as PA not required
9/28/2017	Annual review: Definitions, Background, Guidelines and Recommendations and Evidence-based Scientific References were updated. Updated CC1 and CC3 based on NIAID guidelines.
4/18/2017	Guidelines updated with Medicare Regulations and Coding Guidelines (WPS); updated reference
9/19/2016	Annual review. Literature review did not necessitate any change in coverage criteria
9/18/2015	Initial presentation

β-Hemolytic Streptococcus Testing

Policy Number: AHS – G2159 – β - Hemolytic Streptococcus Testing	Prior Policy Name and Number, as applicable:
Initial Presentation Date: 09/25/2018 Effective Date: February 1, 2025	

Policy Description

Streptococcus are Gram-positive, catalase-negative bacteria that are further divided into α-hemolytic, such as *S. pneumoniae* and *S. mutans*; β-hemolytic, such as *S. pyogenes* (Group A), *S. agalactiae* (Group B), and *S. dysgalactiae subsp equisimilis* (Groups C and G); and γ-hemolytic, such as *Enterococcus faecalis* and *E. faecium* (Wessels, 2024). Streptococcal infections can be manifested in a variety of pathologies, including cutaneous infections, pharyngitis, acute rheumatic fever, pneumonia, postpartum endometritis, and toxic shock syndrome to name a few. Streptococcal infections can be identified using bacterial cultures obtained from blood, saliva, pus, mucosal, and skin samples as well as rapid antigen diagnostic testing (RADT) and nucleic acid-based methodologies (Chow, 2023; Wessels, 2024).

For prenatal screening of Group B Streptococcus, please review policy AHS-G2035.

Related Policies

Policy Number	Policy Title
AHS-G2035	Prenatal Screening (Nongenetic)

Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) For the detection of a streptococcal infection causing respiratory illness, bacterial culture testing from a throat swab **MEETS COVERAGE CRITERIA** when **one** of the following conditions is met:
   a) When the individual has a modified Centor criteria score of 3 or greater (see Note 1 below).
   b) When the individual is suspected of having bacterial pharyngitis in the absence of viral features, (e.g., cough, oral ulcers, rhinorrhea).
   c) Following a negative rapid antigen diagnostic test (RADT) in a symptomatic child or adolescent.

2) Blood culture testing for a streptococcal infection **MEETS COVERAGE CRITERIA** when one of the following conditions is met:
   a) For individuals who fail to demonstrate clinical improvement.
   b) For individuals who have progressive symptoms or clinical deterioration after the initiation of antibiotic therapy.
   c) In cases of suspected prosthetic joint infection.

3) In cases of skin and/or soft tissue infections, bacterial culture testing for a streptococcal infection from a skin swab or from pus **MEETS COVERAGE CRITERIA**.

4) For individuals with suspected acute rheumatic fever (ARF) or post-streptococcal glomerulonephritis (PSGN), the following testing **MEETS COVERAGE CRITERIA**:
   a) Serological titer testing.
   b) Anti-streptolysin O immunoassay.
   c) Hyaluronidase activity or anti-hyaluronidase immunoassay.
   d) Streptokinase activity or anti-streptokinase immunoassay.

5) In cases of suspected viral pharyngitis, bacterial culture testing for streptococci from a throat swab **DOES NOT MEET COVERAGE CRITERIA**.

6) Except in cases of asymptomatic children under the age of three years who have a mitigating circumstance (including a symptomatic family member), RADT for a streptococcal infection **DOES NOT MEET COVERAGE CRITERIA** in any of the following situations:
   a) As a follow-up test for individuals who have had either a bacterial culture test or a nucleic acid test for a streptococcal infection.
   b) As a screening method in an asymptomatic patient.
   c) For individuals with suspected viral pharyngitis.

7) For all situations not described above, serological titer testing **DOES NOT MEET COVERAGE CRITERIA**.

8) Simultaneous ordering of **both** direct probe and amplification probe for the same organism in a single encounter **DOES NOT MEET COVERAGE CRITERIA**.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

9) For all situations not described above, testing with an anti-streptolysin O immunoassay, a hyaluronidase activity or anti-hyaluronidase immunoassay, **or** a streptokinase activity or anti-streptokinase immunoassay **DOES NOT MEET COVERAGE CRITERIA**.

10) For all situations, the following tests **DO NOT MEET COVERAGE CRITERIA**:

   a) Panel tests that screen and identify multiple streptococcal strains (*S. pyogenes* [group A], *S. agalactiae* [group B], *S. dysgalactiae* [groups C/G], ▪-hemolytic streptococcus, and/or ▪-hemolytic streptococcus), using either immunoassay or nucleic acid-based assays (e.g., Solana Strep Complete Assay, Lyra Direct Strep Assay).

   b) MALDI-TOF identification of streptococcus.

   c) The quantification of any strain of streptococcus using nucleic acid amplification, including PCR.

   d) Nicotinamide-adenine dinucleotidase activity or anti-nicotinamide-adenine immunoassay.

NOTES:

Note 1: Centor criteria includes tonsillar exudates, tender anterior cervical lymphadenopathy, fever, and absence of cough with each criterion being worth one point (Chow, 2023).

Table of Terminology

Term	Definition
AAOS	American Academy of Orthopaedic Surgeons
AAP	American Association of Pediatrics
ACOG	American College of Obstetricians and Gynecologists
ADB	Anti-DNase B
AHA	American Heart Association
ARF	Acute rheumatic fever
ASK	Anti-streptokinase
ASM	American Society for Microbiology
ASO	Anti-streptolysin O
ATS	American Thoracic Society
C3	Complement component 3
CAP	Community-acquired pneumonia
CDC	Centers for Disease Control and Prevention
CMS	Centers for Medicare and Medicaid Services
CNS	Central nervous system

CSF	Cerebrospinal fluid
DNA	Deoxyribose nucleic acid
DNases	Deoxyribonucleases
EIA	Enzyme immunoassays
EOS	Early-onset bacterial sepsis
FDA	Food and Drug Administration
GAS	Group A *Streptococcus*
GBS	Group B *Streptococcus*
GCS	Group C *Streptococcus*
GGS	Group G *Streptococcus*
HDA	Helicase-dependent amplification
ICSI	Institute for Clinical Systems Improvement
IDSA	Infectious Diseases Society of America
LDTs	Laboratory developed Tests
LR	Likelihood ratio
MALDI-TOF	Matrix-assisted laser desorption/ionization-Time of flight
NAAT	Nucleic acid amplification test
NADase	Nicotinamide adenine dinucleotidase
NADTs	Rapid antigen detection tests
NICE	National Institute for Health and Care Excellence
OIA	Optical immunoassays
PCR	Polymerase chain reaction
PIDS	Pediatric Infectious Diseases Society
PJI	Prosthetic joint infection
POC	Point of care
PSGN	Post-streptococcal glomerulonephritis
PYR	Pyrrolidonyl aminopeptidase
qPCR	Quantitative PCR
RADT	Rapid antigen diagnostic testing
RIDT	Rapid in vitro diagnostic tests
RNA	Ribonucleic acid
RNATs	Rapid nucleic acid tests
rt-PCR	Real-time polymerase chain reaction
SDSE	*Streptococcus dysgalactiae* subspecies e*quisimilis*
TSA	Trypticase soy agar

Scientific Background

Bacterial acute pharyngitis is caused most often by a Group A Streptococcus (*S. pyogenes* or GAS), accounting for 5-15% of all acute pharyngitis cases in adults. Group C or Group G Streptococcus (*S. dysgalactiae subsp equisimilis* or GCS/GGS) is believed to be a causative agent in 5-10% of the cases of pharyngitis; however, "pharyngitis cause group C or G Streptococcus is clinically indistinguishable from GAS pharyngitis" but is more common in young adults and college students (Chow, 2023). "Diagnosis of infection due to group C streptococci (GCS) and group G streptococci (GGS) depends on identification

of the organism in a culture from a clinical specimen. In general, a positive culture from a normally sterile site, such as blood, synovial fluid, or cerebrospinal fluid (CSF), can be considered definitive evidence of infection in the setting of a compatible clinical syndrome. The interpretation of positive cultures for GCS or GGS from the pharynx or from cutaneous sites such as open ulcers or wounds is less straightforward since asymptomatic colonization of the upper airway and skin also occurs" (Wessels, 2024). GAS occurs most frequently in the very young and the elderly; although, GAS infections can occur in any age-group. The rates of severe GAS infections have been increasing in the United States as well as in other developed nations (Schwartz et al., 1990).

The Centor criteria can be used to gauge the likelihood of pharyngitis due to a GAS infection. The four components of the Centor criteria are tonsillar exudates, tender anterior cervical lymphadenopathy, fever, and absence of cough with each criterion being worth one point. Patients who score less than three according to the Centor criteria are unlikely to have pharyngitis due to GAS and do not require strep testing or antibiotics; patients scoring ≥ three can be tested for GAS pharyngitis (Chow, 2023).

Group A *Streptococcus* is associated with bacterial pharyngitis, scarlet fever, acute rheumatic fever, and post-streptococcal glomerulonephritis. Group A strep pharyngitis presents as a sudden onset of sore throat with odynophagia and fever; it is commonly referred to as "strep throat." In children, additional symptoms can include abdominal pain, nausea, and vomiting. Viral pharyngitis, which accounts for more than 80% of pharyngitis, typically presents with cough, rhinorrhea, hoarseness, oral ulcers, and conjunctivitis unlike GAS pharyngitis. Rare cases of mucopurulent rhinitis caused by GAS has been reported in children under the age of three (CDC, 2024a). Scarlet fever can accompany strep throat. Besides the typical erythematous rash that typically begins on the trunk before spreading outward, scarlet fever can also present as a flushed face, "and the area around the mouth may appear pale (i.e., circumoral pallor)." "Strawberry tongue" can occur due to "yellowish white coating with red papillae" (CDC, 2024b). Scarlet fever is more easily transmitted than asymptomatic carriers through saliva and nasal secretions. Acute Rheumatic Fever (AFR), besides the characteristic fever, can affect the cardiovascular system (carditis and valvulitis), the musculoskeletal system (arthritis), the integumentary system (subcutaneous nodules and erythema marginatum), and the central nervous system (chorea). "Inadequate or lack of antibiotic treatment of streptococcal pharyngitis increases the risk of someone developing acute rheumatic fever. In approximately one-third of patients, acute rheumatic fever follows subclinical streptococcal infections or infections for which medical attention was not sought" (CDC, 2024d). Post-streptococcal glomerulonephritis (PSGN) presents with edema, hypertension, proteinuria, macroscopic hematuria, lethargy, and, at times, anorexia. "Laboratory examination usually reveals mild normocytic normochromic anemia, slight hypoproteinemia, elevated blood urea nitrogen and creatinine, elevated erythrocyte sedimentation rate, and low total hemolytic complement and C3 complement." Urine output is usually decreased, and urine examination "often reveals protein (usually <3 grams per day) and hemoglobin with red blood cell casts" (CDC, 2024c).

The virulence factors of GAS include M proteins, a group of more than 80 known proteins that protein the bacteria against phagocytosis; streptolysin O, a thiol-activated cytolysin; hyaluronidase, which hydrolyzes hyaluronic acid within the host tissue; streptokinase, an enzyme that activates plasmin; nicotinamide-adenine dinucleotidase (NADase), a glycohydrolase of uncertain function; and deoxyribonucleases (DNases) A, B, C, and D. Streptolysin O bind to the eukaryotic membrane's cholesterol to facilitate the characteristic cellular lysis of a GAS infection. Cholesterol and anti-streptolysis O (ASO) antibodies can mitigate streptolysin O damage, and ASO titers often increase following an infection with the peak occurring around four to five weeks post-infection. "Nonsuppurative complications such as rheumatic fever and poststreptococcal glomerulonephritis generally develop during the second or third week of illness… About 80 percent of patients with acute

rheumatic fever or poststreptococcal glomerulonephritis demonstrate a rise in ASO titer; however, the degree of ASO titer elevation does not correlate with severity of disease. In patients with suspected rheumatic fever or glomerulonephritis but with an undetectable ASO titer, prompt testing for other antistreptococcal antibodies such as anti-DNase B (detectable for six to nine months following infection), streptokinase, and antihyaluronidase should be performed" (Stevens & Bryant, 2024).

Acute rheumatic fever (ARF) can occur two to four weeks following GAS pharyngitis. The five major manifestations of ARF are carditis and valvulitis (up to 70% of patients exhibit this condition with ARF), arthritis (up to 66%), CNS system involvement (10-30%), subcutaneous nodules (0-10%), and erythema marginatum (<6%) (Steer & Gibofsky, 2024). A diagnosis of ARF is not predicated by confirmation of a preceding GAS infection; however, it is helpful, especially in diagnosing children and young adults with arthritis and/or carditis. Evidence of GAS should include either a positive throat culture, a positive RADT, or an elevated or rising titer of either ASO or anti-DNase B. These two antibodies are used frequently in clinical practice due to their high sensitivity in diagnosing streptococcal infections (Steer & Gibofsky, 2024; Steer et al., 2015). A study by Blyth and Robertson demonstrated that the sensitivity of using only a single antibody in the diagnosis of streptococcus ranged from 70.5-72.7%; however, the combination of ASO and anti-DNase B increased the specificity to 88.6% with a sensitivity of 95.5%. The addition of anti-streptokinase (ASK) did not increase either the sensitivity or specificity of testing (Blyth & Robertson, 2006).

A study in Norway in 2013 show that necrotizing soft tissue infections can be caused by GAS or GGS/GCS. The mean annual incidence rate is 1.4 per 100,000. During the time period studied (2000-2009), 61 cases of necrotizing soft tissue infections in Norway were due to GAS while nine cases were due to GCS/GGS. "Our findings indicate a high frequency of streptococcal necrotizing fasciitis in our community. GCS/GGS infections contribute to the disease burden but differ from GAS cases in frequency and predisposing factors." They note that "the GCS/GGS patients were older, had comorbidities more often and had anatomically more superficial disease than the GAS patients" (Bruun et al., 2013). A review in 2014 also noted the population most affected by GCS/GGS, but they note that "the case fatality in bacteremia has been reported to be 15-18%" (Rantala, 2014).

Group B Streptococcus (GBS) is frequently found in human gastrointestinal tracts and genitalia and can be spread to the upper respiratory tract of newborns. In neonates, a GBS infections can cause bacteremia, pneumonia, meningitis, and sepsis. GBS can also cause complications in pregnancy, such as urinary tract infections and chorioamnionitis. GBS, in pregnant and postpartum individuals, is of special concern since it is implicated in up to 31% of cases of bacteremia without a focus, eight percent of postpartum endometritis, and two percent of pneumonia; moreover, if left unchecked, GBS can also result in preterm labor and miscarriage. In the adult population at large, GBS infections can be manifest as soft tissue infections, sepsis, and bacteremia (Barshak, 2024; Puopolo et al., 2024). "Invasive disease in infants is categorized on the basis of chronologic age at onset. Early-onset disease usually occurs within the first 24 hours of life (range, 0 through 6 days) and is characterized by signs of systemic infection, respiratory distress, apnea, shock, pneumonia, and less often, meningitis (5%–10% of cases). Late-onset disease, which typically occurs at 3 to 4 weeks of age (range, 7 through 89 days), commonly manifests as occult bacteremia or meningitis (approximately 30% of cases); other focal infections, such as osteomyelitis, septic arthritis, necrotizing fasciitis, pneumonia, adenitis, and cellulitis, occur less commonly. Nearly 50% of survivors of early- or late-onset meningitis have long-term neurologic sequelae (encephalomalacia, cortical blindness, cerebral palsy, visual impairment, hearing deficits, or learning disabilities). Late, late-onset disease occurs at 90 days of age and beyond, usually in very preterm infants requiring prolonged hospitalization" (Pediatrics, 2018).

Type of Testing

Test	Description	Rationale
Culture	Cultures can be taken from a swab of the affected tissue when possible, such as the back of the throat and tonsils (1). The cultures are typically grown on a solid, complex rich medium such as Trypticase Soy Agar (TSA) supplemented with 5% sheep blood so that the zone of b-hemolysis can easily be visualized (2). Culture testing can be supplemented with additional conventional identification tests, such as the Lancefield antigen determination test and the PYR test (3).	The CDC considers the throat culture the 'gold standard' (4). This testing method can be time intensive. "Throat culture also can identify other bacteria that cause pharyngitis less commonly than GAS (eg, group C and group G streptococci, *Arcanobacterium haemolyticum*). However, most laboratories do not routinely identify these pathogens in throat cultures unless specifically requested to do so" (5).
Serology	Many possible serological tests can be performed, including a measurement of the antibody titers associated with a streptococcal infection. Virulence factors that can be monitored include hyaluronidase, streptokinase, nicotinamide-adenine dinucleotidase, DNase B, and streptolysin O. DNase B and streptolysin O are more frequently used in clinical practice (6).	Anti-streptococcal antibody titers represent past infections and should not be used to routinely diagnose an acute infection (7). Antistreptolysin O (ASO) and/or anti-DNase B (ADB) testing can be used to determine prior streptococcal infection associated with disorders such as rheumatic fever and glomerulonephritis. "An increase in titer from acute to convalescent (at least two weeks apart) is considered the best evidence of antecedent GAS infection. The antibody response of ASO peaks at approximately three to five weeks following GAS pharyngitis, which usually is during the first to third week of ARF, while ADB titers peak at six to eight weeks" (8). Antibody titers are dependent on the age of the patients with children having considerably higher 'normal' levels than adults due to frequent exposure to *S. pyrogenes* (3).

Rapid Antigen Diagnostic Testing (RADT)	RADTs can be performed on a swab at the point of care or can be transported to a lab for testing (9). Numerous RADTs directly detect antigens through an agglutination method or the use of immunoassays, including enzyme-based assays, optical assays, and liposome-based assays that are commercially available (3).	Many RADTs are commercially available but can vary considerably in specificity, sensitivity, and ease of use. "In pediatric patients, if the direct antigen test is negative, and if the direct antigen test is known to have a sensitivity of <80%, a second throat swab should be examined by a more sensitive direct NAAT or by culture as a means of arbitrating possible false-negative direct antigen test results. This secondary testing is not necessarily required in adults. A convenient means of facilitating this 2-step algorithm of testing for Streptococcus pyogenes in pediatric patients is to collect a dual swab initially, recognizing that the second swab will be discarded if the direct antigen test is positive" (9).
Nucleic Acid Amplification Tests (NAATs)	NAATs amplify DNA or RNA to detect the presence of microorganisms. Some are offered as point-of-care (POC) rapid diagnostic tests while others require special laboratory equipment (9). Some NAATs utilize real-time polymerase chain reaction (rt-PCR), such as the Lyra Direct Strep Assay, while others use a helicase-dependent amplification (HDA)-based methodology like the Solana Strep Complete assay. NAATs are often qualitative but specific NAATs can be quantitative. NAATs can vary in their selectivity, sensitivity, and ability to differentiate between strains of streptococci.	More sensitive than antibody-based testing for streptococcus. Direct NAATs usually require the use of enriched broth cultures. "Negative direct NAAT results do not have to be arbitrated by a secondary test" (9).
Matrix-Assisted Laser Desorption Ionization-Time	MALDI-TOF mass spectrometry can be used to quickly identify both gram-negative and gram-positive bacteria once the organism is	"For less common organisms, the MALDI-TOF result may not be conclusive, and additional bench tests or molecular tests may be required" (10).

of Flight (MALDI-TOF)	available in a pure culture on solid medium. The results of the MALDI-TOF test are compared to a known database of spectra of microorganisms for identification (10).	
(1) (AACC, 2021);(2) (Gera & McIver, 2013); (3) (Spellerberg & Brandt, 2016); (4) (CDC, 2024a); (5) (Wald, 2024); (6) (Stevens & Bryant, 2024); (7) (Shulman et al., 2012); (8) (Steer & Gibofsky, 2024); (9) (Miller et al., 2018); (10) (Freeman & Roberts, 2023)		

Clinical Utility and Validity

Rapid in vitro diagnostic tests (RIDT), such as the Alere I Strep A, have been CLIA-waived by the FDA. These tests provide results more quickly than the traditional "gold standard" bacterial culture testing. A 2018 study comparing rapid antigen GAS testing, the Alere I Strep A test—an RIDT using isothermal nucleic acid amplification, and throat cultures. "The sensitivity and specificity of the molecular test were 98% and 100%, respectively, compared with culture. There was a 9% false-positive rate with the rapid antigen-based testing…. The Alere test is sufficiently sensitive and specific for definitive GAS testing in a pediatric urgent care setting" (Weinzierl et al., 2018). In Cohen et al. (2016) extensively reviewed the use of rapid antigen detection tests (RADT) for GAS in children. They reviewed 98 unique studies consisting of a total of 101,121 participants and compared both major types of RADTs—enzyme immunoassays (EIA) and optical immunoassays (OIA). "RADT had a summary sensitivity of 85.6%...There was substantial heterogeneity in sensitivity across studies; specificity was more stable. There was no trade-off between sensitivity and specificity….The sensitivity of EIA and OIA tests was comparable (summary sensitivity 85.4% versus 86.2%)… Based on these results, we would expect that amongst 100 children with strep throat, 86 would be correctly detected with the rapid test while 14 would be missed and not receive antibiotic treatment" (Cohen et al., 2016). Another multicenter study using the Alere I Strep A test on cultures obtained from 481 patients of all ages show that the RIDT had 96.0% sensitivity and 94.6% specificity. The authors conclude that this "could provide a one-step, rapid, point-of-care testing method for GAS pharyngitis and obviate backup testing on negative results" (Cohen et al., 2015). This study did note that there are newer tests available that have higher sensitivity, but these tests require more time than the Alere I Strep A method.

Due to the time constraints of clinical laboratories and the variability of RADTs, nucleic acid amplification test (NAAT) use has been increasing in clinical settings. The FDA has approved multiple NAATs for the detection of Streptococcus. The Lyra Direct strep assay is an FDA-approved, NAAT that uses real-time PCR to qualitatively detect the presence of GAS and GGS/GCS in throat swab samples. It should be noted, though, that this assay does not distinguish between GGS and GCS. A study by Boyanton et al. (2016) evaluated the efficacy of the Lyra Direct method as compared to the traditional, time-consuming culture test for GAS and GGS/GCS. The sample sizes were not large (n = 19 for GAS and n = 5 for GGS/GCS out of a total of 161 samples submitted); however, the Lyra Direct strep assay did correctly detect "all b-hemolytic streptococci..." and "in batch mode, the Lyra assay reduced intra-laboratory turnaround time by 60% (18.1 h versus 45.0 h) but increased hands-on time by 96% (3 min 16 s versus 1 min 40 s per specimen)" (Boyanton et al., 2016). The authors note that the RADTs "have largely augmented bacterial culture (the gold standard). However, the performance of commercially available [RADTs] varies greatly depending upon the manufacturer, methodology used (i.e., optical immunoassay, immunochromatographic, or enzyme immunoassay), and the patient population (i.e., pediatric versus adult) being tested. Due to these limitations, nucleic acid amplification tests (NAATs) are being

implemented in clinical laboratories" (Boyanton et al., 2016). The Solana method is also an FDA-approved NAAT, but it uses a rapid helicase-dependent amplification (HDA) methodology. Solana is available for either GAS testing or as a panel testing for GAS, GCS, and GGS. A study by Uphoff et al. (2016) compared the Solana GAS testing to that of conventional culture testing. Their research used 1082 throat swab specimens. The traditional culture tested positive in 20.7% of the samples as compared to 22.6% positive values in the HDA-based methodology. The Solana assay in their results had 98.2% sensitivity and 97.2% specificity. "In 35 min, the HDA method provided rapid, sensitive GAS detection, making culture confirmation unnecessary" (Uphoff et al., 2016). Recently, another study compared an HDA-based method to the Simplex GAS Direct PCR-based method, which is another FDA-approved diagnostic test. The Simplex GAS Direct method does not require initial DNA extraction from the sample, a potential time-saving benefit. The study used 289 throat swabs. The HDA- based method "compared to Simplexa qPCR had sensitivity, specificity, positive predictive value and negative predictive value of 93.1% vs 100%, 100% vs. 100%, 100% vs. 100% and 98.31% vs. 100% respectively… Simplexa qPCR has improved performance and diagnostic efficiency in a high-volume laboratory compared to [HDA-based method] for GAS detection in throat swabs" (Church et al., 2018).

The Solana® Strep Complete Assay by Quidel received FDA clearance in 2016. According to Quidel's FDA application, it is defined as "a rapid in vitro diagnostic test, using isothermal amplification technology (helicase-dependent amplification, HDA) for the qualitative detection and differentiation of Streptococcus pyogenes (Group A β-hemolytic Streptococcus) and Streptococcus dysgalactiae (pyogenic Group C and G β-hemolytic Streptococcus) nucleic acids isolated from throat swab specimens obtained from patients with signs and symptoms of pharyngitis, such as sore throat" (Lollar, 2016). This test must be performed using Quidel's Solana proprietary equipment. According to the 510(k) application, the Solana Strep Complete Assay panel has a clinical sensitivity and specificity for GAS of 98.8% and 98.9%, respectively, as compared to the Lyra Direct Strep Assay's reported 96.5% sensitivity and 98.0% specificity for GAS. The Lyra Direct Strep Assay is a real-time PCR-based assay that cannot differentiate between the pyogenic strains of streptococci. Concerning the pyrogenic GCS/GGS, the Solana Strep Complete Assay panel has a clinical sensitivity of 100% with a specificity of 99.5% as compared to Lyra Direct Strep Assay's reported 95.7% sensitivity and 98.3% specificity for GCS/GGS strains. The reported testing time also varies between the two assays with Solana requiring 25 minutes versus 60-70 minutes for the Lyra Direct Strep Assay (Lollar, 2016).

A recent study by Helmig and Gertsen (2017) evaluated the accuracy of PCR-based testing for GBS in pregnant individuals. Their study used rectovaginal swabs from 106 women in gestational weeks 35-37. For each, both a GBC culture and a PCR-based molecular GBS test (Xpert GBS of Cepheid Ltd) were performed. Only one PCR test yielded no result, so the invalid PCR-based test rate is <1%. There were 25/106 of the GBS cultures tested positive as compared to 27/105 of the PCR-based test. The specificity of the PCR-based test was 97.5% with a 100% sensitivity and a 92.6% positive predictive value. The authors conclude that "the PCR test has sufficient accuracy to direct intrapartum antibiotic prophylaxis for GBS transmission during delivery" (Helmig & Gertsen, 2017). A preliminary study in France of 1416 mothers with newborns compared swab cultures and GBS PCR assay for their predictive value of early-onset bacterial sepsis (EOS) in newborns since GBS is the most common cause of EOS. The results show that "the diagnostic values of the two tests highlighted a nonsignificant superiority of intrapartum GBS PCR assay" but that "the negative predictive value was improved with intrapartum PCR assay (negative likelihood ratio [LR]: 0.3 [0.1-0.9] vs. 0.6 [0.4-1.1])…. These results suggest that the intrapartum GBS PCR assay offers a better predictive value of GBS EOS that the usual vaginal culture swab at the 9th month but requires confirmation by large studies" (Raignoux et al., 2016).

Luo et al. (2019) "evaluated the overall diagnosis and treatment of acute pharyngitis in the United States, including predictors of test type and antibiotic prescription." Five categories of tests were identified, which were RADT [rapid antigen detection test], RADT plus culture, other tests, nucleic acid amplification testing (NAAT), and no test. Pharyngitis events from 2011-2015 were examined and a total of 18.8 million pharyngitis events across 11.6 million patients were included. Overall, 68.2% of events were found to occur once, with 29.1% requiring further follow-up. Furthermore, 43% of events were diagnosed by RADT and 20% were diagnosed by RADT plus culture. NAAT testing also increased 3.5-fold from 2011-2015 (going from 0.06% to 0.27%). Antibiotics were used in 49.3% of events as a whole. For RADT plus culture, antibiotics were used 31.2% of the time, for NAAT alone, 34.5%, for RADT alone, 54.2%, for no test, 57.1%. The authors concluded that "Diagnostic testing can help lower the incidence of inappropriate antibiotic use, and inclusion of NAAT in the clinical guidelines for GAS pharyngitis warrants consideration" (Luo et al., 2019).

O. Luiz et al. (2019) evaluated the "prevalence and persistence of beta-haemolytic streptococci throat carriage and type the bacterial population." A total of 121 children and 127 young adult volunteers contributed throat swabs (for culture), and these volunteers were screened quarterly for beta-haemolytic bacterial species. Carriage was detected in 34 volunteers (13.7%). Seventeen children were found to carry Group A *Streptococcus*, while seventeen young adults were found to carry four separate subspecies (*Streptococcus dysgalactiae* subsp. *equisimilis* (SDSE), *Streptococcus pyogenes*, *Streptococcus agalactiae* and the *Streptococcus anginosus* group). The authors also identified persistent carriage for as long as six months in two children and for as long as one year in three young adults. The authors concluded that "prevalence was slightly greater among children, but persistent carriage was greater among young adults, with SDSE being the species most associated with persistence" (O. Luiz et al., 2019).

Fraser et al. (2020) performed a meta-analysis to assess the cost-effectiveness of point-of-care testing for detection of Group A Streptococcus. The authors remarked that this type of testing has seen increased use as an adjunct for managing care, such as for prescribing antibiotics. Thirty-eight studies of clinical effectiveness were included, along with three studies of cost-effectiveness. Twenty-six articles "reported on the test accuracy of point-of-care tests and/or clinical scores with biological culture as a reference standard." Overall, 21 point-of-care tests were evaluated. The authors identified two populations of interest; "patients with Centor/McIsaac scores of ≥ 3 points or FeverPAIN scores of ≥ 4 points." Test sensitivity for these populations ranged from 0.829-0.946 while test specificity ranged from 0.849-0.991. However, the authors did note there was significant heterogeneity and expressed doubts that any single study "accurately captured a test's true performance." The authors developed an economic model to explore the cost-effectiveness of this type of testing, and 14 of the 21 tests were included in this model. Per the current National Institute for Health and Care Excellence's cost-effectiveness thresholds, these tests were not found to be cost-effective. The authors acknowledged significant uncertainties in the estimates, such as penalties for antibiotic over-prescriptions. The authors concluded that "the systematic review and the cost-effectiveness models identified uncertainties around the adoption of point-of-care tests in primary and secondary care settings. Although sensitivity and specificity estimates are promising, we have little information to establish the most accurate point-of-care test" (Fraser et al., 2020; Kim et al., 2019).

Bilir et al. (2021) studied the cost-effectiveness of point of care (POC) nucleic acid amplification tests (NAAT) for streptococcus in the US. Point of care NAAT was compared to rapid antigen detection tests (RADT) and culture. Costs, clinical effects, antibiotic complications, number of patients treated, and antibiotic utilization were studied. Analysis showed that the POC NAAT method would cost $44 per patient while RADT and culture would cost $78 per patient. "Compared with RADT + culture, POC NAAT would increase the number of appropriately treated patients and avert unnecessary use of antibiotics."

According to the results, "POC NAAT would be less costly and more effective than RADT + culture; POC NAAT adoption may yield cost savings to US third-party payers. Access to POC NAAT is important to optimize GAS diagnosis and treatment decisions in the United States" (Bilir et al., 2021).

In a metanalysis, Dubois et al. (2021) studied the diagnostic accuracy of rapid antigen detection tests (NADTs) vs rapid nucleic acid tests (RNATs) for diagnosis of group A streptococcal pharyngitis. A total of 38 studies using RNAT were included, with a sensitivity of 97.5% and specificity of 95.1%. RADTs had a sensitivity of 82.3%, but specificity was similar to the sensitivity of RNATs. Overall, RNATs were more sensitive than RADTs. The authors conclude that "the high diagnostic accuracy of RNATs may allow their use as stand-alone tests to diagnose group A streptococcus pharyngitis" (Dubois et al., 2021).

McCarty et al. (2022) studied the clinical utility on the GenMark Dx ePlex® blood culture identification gram-positive panel. The panel results were evaluated and compared to MALDI-TOF mass spectrometry and traditional antimicrobial susceptibility testing. One hundred Gram-Positive bacteria were represented. "The positive percent agreement (PPA) was 97/97 with 2 false positives." The study included chart reviews of 80 patients. The average time for organism identification was 24.4 hours faster, and the average time for optimization was 29.2 hours faster for the eight patients identified with organisms such as streptococci. The authors "confirm high sensitivity and specificity of the FDA-cleared GenMark Dx ePlex BCID-GP Panel compared to MALDI-TOF MS on bacterial isolates and identify opportunities for earlier optimization of antimicrobial therapy that may also be accompanied by potential cost savings (McCarty et al., 2022).

Guidelines and Recommendations

Centers for Disease Control and Prevention

Acute Pharyngitis (CDC, 2024a): Most cases of acute pharyngitis are viral. Only 20-30% of pharyngitis episodes in children and 5-15% in adults are due to group A *Streptococcus* (GAS). History and clinical examination can be used to diagnosis viral pharyngitis when clear viral symptoms (e.g., cough, rhinorrhea, hoarseness, oral ulcers, conjunctivitis) are present; these patients do not need testing for group A strep. However, clinical examination cannot be used to differentiate viral and group A strep pharyngitis in the absence of viral symptoms, even for experienced clinicians. The diagnosis of group A strep pharyngitis is confirmed by either a rapid antigen detection test (RADT) or a throat culture. RADTs have high specificity for group A strep but varying sensitivities when compared to throat culture, which is considered the gold standard diagnostic test. Healthcare providers can use a positive RADT or throat culture as confirmation of group A strep pharyngitis. For children older than three years old healthcare providers should follow up a negative RADT with a throat culture. For all other ages a throat culture after a negative RADT is not routinely indicated (CDC, 2024a).

Scarlet Fever (CDC, 2024b): Scarlet fever (scarlatina) consists of an erythematous rash caused by GAS and can occur along with acute pharyngitis. "The differential diagnosis of scarlet fever with pharyngitis includes multiple viral pathogens that can cause acute pharyngitis with a viral exanthema." To confirm scarlet fever with pharyngitis, healthcare providers need to use either a rapid antigen detection test (RADT) or throat culture. RADTs have high specificity for group A strep but varying sensitivities when compared to throat culture. Throat culture is the gold standard diagnostic test. Clinicians should follow up a negative RADT in children older than three with symptoms of scarlet fever with a throat culture. Clinicians should have a mechanism in place to contact the family and initiate antibiotics if the back-up throat culture is positive (CDC, 2024b).

Post-Streptococcal Glomerulonephritis (PSGN) (CDC, 2024c): PSGN is primarily due to a GAS infection, but rare cases of GCS-induced PSGN have been reported. Clinical features include edema, hypertension, proteinuria, macroscopic hematuria, and lethargy. As such, "The differential diagnosis of PSGN includes other infectious and non-infectious causes of acute glomerulonephritis. Clinical history and findings with evidence of a preceding group A strep infection should inform a PSGN diagnosis. Evidence of preceding group A strep infection can include

- Isolation of group A strep from the throat
- Isolation of group A strep from skin lesions
- Elevated streptococcal antibodies" (CDC, 2024c).

Acute Rheumatic Fever (CDC, 2024d): "The differential diagnosis of acute rheumatic fever is broad due to the various symptoms of the disease. The differential diagnosis may include specific autoimmune diseases, inflammatory diseases, cancers, and other conditions." The CDC notes that no definitive diagnostic test exists for acute rheumatic fever and recommends using the Jones criteria (endorsed by the American Heart Association) to make a clinical diagnosis, which now includes the addition of subclinical carditis as a major manifestation for low, moderate, and high risk populations" (CDC, 2024d).

American Association of Pediatrics (AAP)

The AAP has published the Red Book (Kimberlin et al., 2021) as guidance for infectious diseases in the pediatric population. Their relevant comments and recommendations include:

- "Children with pharyngitis and obvious viral symptoms (eg, rhinorrhea, cough, hoarseness, oral ulcers) should not be tested or treated for GAS [Group A Streptococcus] infection; testing also generally is not recommended for children younger than 3 years."
- "Several rapid diagnostic tests for GAS pharyngitis are available…Specificities of these tests generally are high (very few false-positive results), but the reported sensitivities vary considerably (ie, false-negative results occur)."
- "The US Food and Drug Administration (FDA) has cleared a variety of rapid tests for use in home settings. Parents should be informed that home use is discouraged because of the risk of false-positive testing that represents colonization."
- "Because of the very high specificity of rapid tests, a positive test result does not require throat culture confirmation. Rapid diagnostic tests using techniques such as polymerase chain reaction (PCR), chemiluminescent DNA probes, and isothermal nucleic acid amplification tests have been developed…Some studies suggest that these tests may be as sensitive as standard throat cultures on sheep blood agar."
- "Children with manifestations highly suggestive of viral infection, such as coryza, conjunctivitis, hoarseness, cough, anterior stomatitis, discrete ulcerative oral lesions, or diarrhea, are very unlikely to have true GAS pharyngitis and should not be tested."
- "Testing children younger than 3 years generally is not indicated. Although small outbreaks of GAS pharyngitis have been reported in young children in child care settings, the risk of ARF is so remote in young children in industrialized countries that diagnostic studies for GAS pharyngitis generally are not indicated for children younger than 3 years."
- "In contrast, children with acute onset of sore throat and clinical signs and symptoms such as pharyngeal exudate, pain on swallowing, fever, and enlarged tender anterior cervical lymph nodes, without concurrent viral symptoms and/or exposure to a person with GAS pharyngitis, are more likely to have GAS infection and should have a rapid antigen test and a throat culture if the rapid test result is negative, with treatment initiated if a test result is positive."

- "Testing asymptomatic household contacts for GAS infection is not recommended except when the contacts are at increased risk of developing sequelae of GAS infection, such as ARF or acute glomerulonephritis; if test results are positive, such contacts should be treated."
- "Testing asymptomatic household contacts usually is not helpful. However, if multiple household members have pharyngitis or other GAS infections, simultaneous cultures of all household members and treatment of all with positive cultures or rapid antigen test results may be of value."
- "In suspected invasive GAS infections, cultures of blood and of focal sites of possible infection are indicated."
- "Laboratory evidence of antecedent GAS infection should be confirmed in all cases of suspected ARF [acute rheumatic fever], and evidence includes an increased or rising ASO or anti-DNAase B titer, or a positive rapid antigen or streptococcal throat culture. Because of the long latency between GAS infection and presentation with chorea, such laboratory evidence may be lacking in cases where chorea is the major criteria."
- "Post-treatment throat swab cultures are indicated only for patients who are at particularly high risk of ARF [acute rheumatic fever] (eg, those living in an area with endemic infection)."

Regarding the management of infants at risk of group B streptococcal disease, a list of recommendations was provided. The relevant points are included below:

- "Early-onset GBS infection is diagnosed by blood or CSF culture. Common laboratory tests such as the complete blood cell count and C-reactive protein do not perform well in predicting early-onset infection, particularly among well-appearing infants at lowest baseline risk of infection."
- "Evaluation for late-onset GBS disease should be based on clinical signs of illness in the infant. Diagnosis is based on the isolation of group B streptococci from blood, CSF, or other normally sterile sites. Late-onset GBS disease occurs among infants born to mothers who had positive GBS screen results as well as those who had negative screen results during pregnancy. Adequate IAP does not protect infants from late-onset GBS disease" (Puopolo et al., 2019).

American Heart Association (AHA)

The AHA published a revision to the Jones criteria for diagnosis of acute rheumatic fever in 2015. In it, they note the importance of identifying laboratory evidence of a group A streptococcal infection. The AHA lists three clinical features that can serve as evidence for a preceding Group A Streptococcus infection, which are as follows:

- "Increased or rising anti-streptolysin O titer or other streptococcal antibodies (anti-DNASE B). A rise in titer is better evidence than a single titer result."
- "A positive throat culture for group A β-hemolytic streptococci."
- "A positive rapid group A streptococcal carbohydrate antigen test in a child whose clinical presentation suggests a high pretest probability of streptococcal pharyngitis" (Gewitz et al., 2015).

Institute for Clinical Systems Improvement (ICSI)

In 2017, the ICSI updated their guidelines titled *Diagnosis and treatment of respiratory illness in children and adults*. They give the following consensus recommendation: "It is the consensus of the ICSI work group to NOT test for Group A Streptococcal (GAS) pharyngitis in patients with modified Centor criteria scores less than three or when viral features like rhinorrhea, cough, oral ulcers and/or hoarseness are present. Testing should generally be reserved for patients when there is a high suspicion for GAS and for whom there is intention to treat with antibiotics" (Short et al., 2017). The Centor criteria include age of

patient, physical state of the tonsils and lymph nodes, temperature, and presence or absence of cough (Centor & McIsaac, 2024).

American Thoracic Society (ATS) and Infectious Diseases Society of America (IDSA)

The ATS and IDSA published a joint guideline on the diagnosis and treatment of community-acquired pneumonia in adults. The guideline notes that group A *Streptococcus* may be associated with influenza pneumonia. Their relevant recommendations are listed below:

- "We recommend not obtaining sputum Gram stain and culture routinely in adults with CAP managed in the outpatient setting (strong recommendation, very low quality of evidence)."
- "We recommend not obtaining blood cultures in adults with CAP managed in the outpatient setting (strong recommendation, very low quality of evidence)" (Metlay et al., 2019).

Infectious Diseases Society of America (IDSA)

The 2014 update of the IDSA's guidelines concerning skin and soft tissue infections included a recommendation (strong; moderate-quality evidence) of "Gram stain and culture of the pus or exudates from skin lesions of impetigo and ecthyma are recommended to help identify whether *Staphylococcus aureus* and/or ▪-hemolytic *Streptococcus* is the cause, but treatment without these studies is reasonable in typical cases." They make a similar recommendation in the cases of pus from carbuncles and abscesses as well as pyomyositis; however, they do not recommend (strong, moderate) a "Gram stain and culture of pus from inflamed epidermoid cysts." As for erysipelas and cellulitis, "cultures of blood or cutaneious aspirates, biopsies, or swabs are not routinely recommended (strong, moderate) …cultures of blood are recommended (strong, moderate), and cultures and microscopic examination of cutaneious aspirates, biopsies, or swabs should be considered in patients with malignancy on chemotherapy, neutropenia, severe cell-mediated immunodeficiency, immersion injuries, and animal bites (weak, moderate)" (Stevens et al., 2014).

The IDSA and the American Society for Microbiology (ASM) published a guideline in 2018 titled "A Guide to Utilization of the Microbiology Laboratory for Diagnosis of Infectious Diseases." This guideline includes items on the laboratory diagnosis of pharyngitis, which are as follows:

- For *Streptococcus pyogenes*, direct NAAT, nucleic acid probe tests, or a rapid direct antigen test (followed by a culture or NAAT test if negative) may all be performed.
- For Groups C and G β-hemolytic streptococci, a NAAT may be performed, or a combination of throat culture and antigen tests on isolates for groups C and G streptococci may be performed.

Other relevant comments include:

- "A rapid antigen test for *Streptococcus pyogenes* may be performed at the point of care by healthcare personnel or transported to the laboratory for performance of the test…in pediatric patients, if the direct antigen test is negative, and if the direct antigen test is known to have a sensitivity of <80%, a second throat swab should be examined by a more sensitive direct NAAT or by culture as a means of arbitrating possible false-negative direct antigen test results…this secondary testing is not necessarily required in adults"
- "Direct and amplified NAATs for *Streptococcus pyogenes* are more sensitive than direct antigen tests and, as a result, negative direct NAAT results do not have to be arbitrated by a secondary test."

- "Detection of group C and G β-hemolytic streptococci is accomplished by throat culture in those patients in whom there exists a concern for an etiologic role for these organisms. Only large colony types are identified, as tiny colonies demonstrating groups C and G antigens are in the *Streptococcus anginosus (S. milleri)* group" (Miller et al., 2018).

American Academy of Otolaryngology-Head and Neck Surgery Foundation

Although the focus of this guideline is the tonsillectomy procedure in children, there are some relevant comments. The Academy notes that "In practice, streptococcal carriage is strongly suggested by positive strep cultures or other strep tests when the child lacks signs or symptoms of acute pharyngitis" (Mitchell et al., 2019). IDSA endorsed this guideline in February 2019 (IDSA, 2019a).

American Academy of Orthopaedic Surgeons

Although this guideline focuses on management of periprosthetic joint infections, there is a relevant recommendation, which states that "synovial fluid aerobic and anaerobic bacterial cultures" have moderate evidence to support their use to "aid in the diagnosis of prosthetic joint infection (PJI)" (AAOS, 2019). IDSA endorsed this guideline in March 2019 (IDSA, 2019b).

2011 Pediatric Infectious Diseases Society (PIDS) and Infectious Diseases Society of America (IDSA)

The 2011 joint PIDS-IDSA guidelines concerning pediatric community-acquired pneumonia (CAP) recommended (strong recommendation; moderate-quality evidence) that "blood cultures should not be routinely performed in nontoxic, fully immunized children with CAP managed in the outpatient setting" and that "blood cultures should be obtained in children who fail to demonstrate clinical improvement and in those who have progressive symptoms or clinical deterioration after initiation of antibiotic therapy." Concerning inpatient services, they recommend (strong recommendation; low-quality evidence) that "blood cultures should be obtained in children requiring hospitalization for presumed bacterial CAP that is moderate to severe, particularly those with complicated pneumonia"; however, "in improving patients who otherwise meet criteria for discharge, a positive blood culture with identification or susceptibility results pending should not be routinely preclude discharge of that patient with appropriate oral or intravenous antimicrobial therapy. The patient can be discharged if close follow-up is assured (weak recommendation; low-quality evidence)." For pneumococcal bacteremia, they do not recommend repeated blood cultures to document resolution (weak recommendation; low-quality evidence), but they do recommend "repeated blood cultures to document resolution of bacteremia…caused by *S. aureus*, regardless of clinical status (strong recommendation; low-quality evidence)." With respect to sputum gram stain and culture, "sputum samples for culture and Gram stain should be obtained in hospitalized children who can produce sputum" (weak recommendation; low-quality evidence). They do not recommend using urinary antigen detection testing "for the diagnosis of pneumococcal pneumonia in children; false-positive tests are common (strong recommendation; high-quality evidence)" (Bradley et al., 2011).

American College of Obstetricians and Gynecologists (ACOG)

The ACOG issued Committee Opinion #797 in 2020. ACOG recommends that "Regardless of planned mode of birth, all pregnant women should undergo antepartum screening for GBS at 36 0/7–37 6/7 weeks of gestation, unless intrapartum antibiotic prophylaxis for GBS is indicated because of GBS bacteriuria during the pregnancy or because of a history of a previous GBS-infected newborn" (ACOG, 2020). This committee opinion was reaffirmed in 2022.

American Society for Microbiology

The ASM endorsed the above ACOG recommendation, stating that "The recommended screening interval has changed from 35-37 weeks (per CDC 2010 guidelines) to 36 0/7 to 37 6/7 weeks (ACOG 2019 recommendations)." Concerning identification of group B *streptococcus*, the ASM propounds the following:

"Recommendation: Acceptable phenotypic and proteomic methods of identification of candidate isolates include CAMP test, latex agglutination, and mass spectrometry."

"Recommendation: Nucleic acid amplification-based identification of GBS from enrichment broth is acceptable, but not sufficient for all patients."

"Recommendation: Latex agglutination directly from enrichment broth and direct-from-specimen immunoassays are unacceptable methods for GBS detection."

The guideline also recommends performing "antimicrobial susceptibility testing on all GBS [Group B Streptococcus] isolates from pregnant women with penicillin allergy", and most recently the ASM included options for vancomycin reporting (Filkins et al., 2021).

National Institute for Health and Care Excellence

The NICE published an update on "rapid tests for group A streptococcal infections in people with a sore throat." They stated that "Rapid tests for strep A infections are not recommended for routine adoption for people with a sore throat. This is because their effect on improving antimicrobial prescribing and stewardship, and on patient outcomes, as compared with clinical scoring tools alone, is likely to be limited" (NICE, 2019).

Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

The FDA approved the Lyra Direct Strep Assay (k133833) on 04/16/2014 and reclassified it on 07/11/2014. It is a "Real-Time PCR *in vitro* diagnostic test for the qualitative detection and differentiation of Group A β -hemolytic *Streptococcus* (*Streptococcus pyogenes*) and pyogenic Group C and G β -hemolytic *Streptococcus* nucleic acids isolated from throat swab specimens obtained from patients with signs and symptoms of pharyngitis, such as sore throat. The assay does not differentiate between pyogenic Groups C and G β-hemolytic *Streptococcus*" (Hojvat, 2014). The FDA has also approved the Solana Strep Complete Assay by Quidel that is "an in vitro diagnostic test for the detection of Group A, C

and G beta- hemolytic *Streptococcus* in throat swab specimens from symptomatic patients" on 10/25/2016 (K162274) (FDA, 2016).

On 03/06/2019, the FDA approved GenePOC's Strep A assay to be performed using GenePOC's Revogene instrument as a "single-use test for qualitative detection of *Streptococcus pyogenes* (group A *Streptococcus*-GAS) nucleic acids from throat swab specimens obtained from patients with signs and symptoms of pharyngitis" (FDA, 2019).

On November 9, 2020, the FDA approved Mesa Biotech, Inc.'s Accula™ Strep A Test, which is a semi-automated, colorimetric polymerase chain reaction (PCR) nucleic acid amplification test "to qualitatively detect Streptococcus pyogenes (Group A βhemolytic Streptococcus, Strep A) bacterial nucleic acid from unprocessed throat swabs that have not undergone prior nucleic acid extraction" (FDA, 2020).

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
86060	Antistreptolysin 0; titer
86063	Antistreptolysin 0; screen
86215	Deoxyribonuclease, antibody
86317	Immunoassay for infectious agent antibody, quantitative, not otherwise specified
86318	Immunoassay for infectious agent antibody(ies), qualitative or semiquantitative, single step-method (eg, reagent strip);
87040	Culture, bacterial; blood, aerobic, with isolation and presumptive identification of isolates (includes anaerobic culture, if appropriate)
87070	Culture, bacterial; any other source except urine, blood or stool, aerobic, with isolation and presumptive identification of isolates
87071	Culture, bacterial; quantitative, aerobic with isolation and presumptive identification of isolates, any source except urine, blood or stool
87077	Culture, bacterial; aerobic isolate, additional methods required for definitive identification, each isolate
87081	Culture, presumptive, pathogenic organisms, screening only;
87430	Infectious agent antigen detection by immunoassay technique, (eg, enzyme immunoassay [EIA], enzyme-linked immunosorbent assay [ELISA], fluorescence immunoassay [FIA], immunochemiluminometric assay [IMCA]) qualitative or semiquantitative; Streptococcus, group A
87650	Infectious agent detection by nucleic acid (DNA or RNA); Streptococcus, group A, direct probe technique
87651	Infectious agent detection by nucleic acid (DNA or RNA); Streptococcus, group A, amplified probe technique
87652	Infectious agent detection by nucleic acid (DNA or RNA); Streptococcus, group A, quantification

87797	Infectious agent detection by nucleic acid (DNA or RNA), not otherwise specified; direct probe technique, each organism
87798	Infectious agent detection by nucleic acid (DNA or RNA), not otherwise specified; amplified probe technique, each organism
87799	Infectious agent detection by nucleic acid (DNA or RNA), not otherwise specified; quantification, each organism
87880	Infectious agent antigen detection by immunoassay with direct optical (ie, visual) observation; Streptococcus, group A

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

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Revision History

Revision Date	Summary of Changes
09/04/2024	Reviewed and Updated: Updated background, guidelines, and evidence-based scientific references. Literature review necessitated the following changes in coverage criteria: Updated CC6a to include nucleic acid testing, now reads: "a) As a follow-up test for individuals who have had either a bacterial culture test or a nucleic acid test for a streptococcal infection."

Biochemical Markers of Alzheimer Disease and Dementia

Policy Number: AHS – G2048 – Biochemical Markers of Alzheimer Disease and Dementia	Prior Policy Name and Number, as applicable: • AHS – G2048 – Biochemical Markers of Alzheimer's Disease
Initial Presentation Date: 09/18/2015 Revision Date: 2/1/2025	

Policy Description

Alzheimer disease (AD) is a neurodegenerative disease defined by a gradual decline in memory, cognitive functions, gross atrophy of the brain, and accumulation of extracellular amyloid plaques and intracellular neurofibrillary tangles (Karch et al., 2014).

Related Policies

Policy Number	Policy Title
AHS-M2038	Genetic Testing for Familial Alzheimer Disease

Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in "Applicable State and Federal Regulations" section of this policy document.

1) For individuals with Alzheimer disease or mild cognitive impairment, measurement of amyloid beta peptides in cerebrospinal fluid **MEETS COVERAGE CRITERIA**.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

2) Measurement of cerebrospinal fluid biomarkers of Alzheimer disease or dementia not mentioned above (e.g., tau protein, α-synuclein, or neural thread proteins) **DOES NOT MEET COVERAGE CRITERIA**.

3) Measurement of plasma and/or serum biomarkers of Alzheimer disease or dementia (e.g., tau protein, amyloid beta peptides, neural thread proteins, ApoE, and ApoE4) **DOES NOT MEET COVERAGE CRITERIA**.

4) Measurement of urinary biomarkers of Alzheimer disease or dementia (e.g., neural thread proteins, amyloid beta peptides, and urinary extracellular vesicle analysis) **DOES NOT MEET COVERAGE CRITERIA**.

5) The use of multianalyte assays, algorithmic analysis, and/or any other tests not mentioned above for the prognosis, diagnosis, and/or management of Alzheimer disease or dementia **DOES NOT MEET COVERAGE CRITERIA**.

Table of Terminology

Term	Definition
AAN	American Academy of Neurology
AD	Alzheimer disease
AD7c-NTP	Alzheimer-associated neuronal thread protein
ADAD	Autosomal dominant Alzheimer's disease
ALS	Amyotrophic lateral sclerosis
aMCI	Amnestic mild cognitive impairment
APOE	Apolipoprotein E
APOJ	Apolipoprotein J
Aß40	Amyloid Beta 40
Aß42	Amyloid Beta 42
AUC	Area under the curve
Aβ 25-35	B-amyloid 25-35
CCCDTD	Canadian Consensus Conference on the Diagnosis and Treatment of Dementia
CDC	Centers For Disease Control and Prevention
CLIA '88	Clinical Laboratory Improvement Amendments Of 1988
CMS	Centers For Medicare and Medicaid
CN	Cognitively normal
CNT	Carbon nanotubes
CSF	Cerebrospinal fluid
CT	Computerized tomography
CU	Cognitively unimpaired
DLB	Dementia with lewy bodies

EFNS	European Federation of Neurological Societies
EV	Extracellular vesicle
FDA	Food And Drug Administration
FDG	Fluoro-deoxyglucose
GBSC	Global Biomarker Standardization Consortium
GSEA	Gene set analysis
HD	Huntington disease
IWG	International Working Group
JPND	Joint Program—Neurodegenerative Disease Research
LDT	Laboratory-developed test
LP	Lumbar puncture
MCI	Mild cognitive impairment
MRI	Magnetic resonance imaging
NFH	Heavy chain
NFL	Neurofilament light chain
NG	Neurogranin
NIA	National Institute on Aging
NICE	National Institute for Health and Care Excellence
NINCDS	National Institute of Neurological and Communicative Disorders and Stroke
NrCAM	Neuronal cell adhesion molecule
NTP	Neuronal thread protein
PD	Parkinson disease
PET	Positron emission tomography
PKCe	Protein kinase c-epsilon
P-tau	Phosphorylated tau
PTP	Pancreatic thread protein
REM	Rapid eye movement
SCD	Subjective cognitive decline
sCJD	Sporadic Creutzfeldt–Jakob disease
SNAP23	Synaptosomal-associated protein 23
SORL	Sortilin-related receptor
T-tau	Total tau
USPSTF	United States Preventive Services Task Force

Scientific Background

Alzheimer disease (AD) is a devastating neurodegenerative disease with a strong genetic component and is the predominant form of dementia (60-70%). As of March, 2023, more than 55 million people worldwide were living with dementia and it is the seventh leading cause of death and globally is one of the major causes of disability and dependency among older individuals(WHO, 2023). The average lifetime risk of developing AD is 10–12%; this risk at least doubles with the presence of a first-degree relative with the disorder (Goldman et al., 2011). The genetic predisposition of AD, even for late-onset AD patients, is estimated to be 60–80% (Gatz et al., 2006). According to the Centers for Disease Control

and Prevention (CDC), the total adjusted death rates in the U.S. varied according to ethnicity with white, non-Hispanics having a rate of 70.8 per 100,000 individuals as compared to 65.0 and 46.0 per 100,000 for non-Hispanic black and Hispanic individuals (Kramarow & Tejada-Vera, 2019).

Most patients develop clinical symptoms at or after the age of 65 (spontaneous or late-onset AD), however 2–10% of patients have an earlier onset of disease (early-onset AD) (Shea et al., 2016). AD is characterized by severe neuronal loss, aggregation of extracellular amyloid β plaques, and intraneuronal tau protein tangles, resulting in progressive deterioration of memory and cognitive functions and ultimately requiring full-time medical care (Sala Frigerio & De Strooper, 2016). There is an enormous burden on public health due to the high costs associated with care and treatment. Aside from drugs that temporarily relieve symptoms, no treatment exists for AD (Van Cauwenberghe et al., 2016).

Many genetic studies have recently identified that late-onset Alzheimer disease is associated with the apolipoprotein E (*APOE*), apolipoprotein J (*APOJ*), and sortilin-related receptor (*SORL*) genes mainly expressed by various types of glial cells such as microglia, oligodendrocytes, and astrocytes; this has helped AD-related research stray from neurons and toward glial cells and neuroinflammation (Arranz & De Strooper, 2019).

The pathological processes of AD and other degenerative dementias are likely well underway before clinical symptoms manifest, therefore, biomarkers may have potential utility in the early diagnosis of dementia (Peterson, 2024). Mild cognitive impairment (MCI) is an intermediate state between normal cognition and dementia, recognizable as an early manifestation of dementia. MCI due to AD is the most common type of MCI (Bennett et al., 2002).

Studies have examined the use of cerebrospinal fluid (CSF) markers for predicting conversion from MCI to dementia. The most replicated CSF biomarkers include tau protein or phosphorylated tau protein and amyloid beta 42 (Aß42) peptide, which may be represented by a low ratio of Aß42 to Aß40 levels, or a low ratio of Aß42 to tau levels. However, these tests vary in sensitivity (36 to 100 percent) and specificity (29 to 91 percent), and in the types of assays used. Recent research notes that the Aß42/40 ratio should be used over the measurement of Aß42 alone, as this ratio gives a more accurate diagnosis when analyzing CSF AD biomarkers (Hansson et al., 2019). Currently, these markers are of marginal clinical utility and do not have an established role in the evaluation of patients in the clinical setting (Peterson, 2024; Wolk & Dickerson, 2024).

Other biomarkers in CSF such as cargo proteins (e.g. chromogranin-B, α-synuclein), carnosinase I, chromogranin A, and NrCAM (neuronal cell adhesion molecule) have been proposed to provide clinical value for assessment of AD. Levels of each of the above CSF proteins are found to be statistically different among clinically defined patient groups with different degrees of cognitive impairment. However, the absence of a clinical treatment makes this relatively invasive test of questionable clinical utility (Schaffer et al., 2015; Wolk & Dickerson, 2024).

Plasma levels of the E4 variant of apolipoprotein E (ApoE4) may be a less invasive option for diagnosing patients. *ApoE* facilitates the delivery of cholesterol and promotes neuronal functionality and decreased apoE4 levels associated with neuronal degradation are suggestive of AD (Farrer et al., 1997). However, results are inconsistent across various studies. The correlation between altered levels of *ApoE* and ApoE4 with AD pathology is still not definitive, and standardization of methods is needed (Schaffer et al., 2015).

Studies have been conducted comparing the telomere length of peripheral blood leukocytes with those in the cerebellum (Patel et al., 2011). The shortening of telomere length is indicative of chronic stress on

the human body, common in AD patients. However, cerebellar telomere length is not considered a diagnostic tool to evaluate the risk of inherited AD (Patel et al., 2011). Moreover, many other diseases also contain pathologies that induce stress on the body, so results may be confounded with other underlying health problems (Schaffer et al., 2015).

High concentrations of neuronal thread protein (NTP), specifically AD-associated NTP (AD7c-NTP), in urine is found to be representative of AD pathology (Patel et al., 2011). NTP is a brain protein that interacts with antibodies produced against pancreatic thread protein (PTP), a protein that contains structural components highly similar to the fibrils found in neuronal plaques in AD patients (Blennow et al., 2012; Patel et al., 2011). Moreover, AD7c-NTP is reflective of neuronal cell dysfunction. Unfortunately, NTP is more useful in determining the progression of the disease in patients who already have AD and not for early diagnosis (Lonneborg, 2008; Schaffer et al., 2015).

Studies have also identified a potential relationship between nanoscale extracellular vesicles (exosomes) and AD. Researchers note that exosomes may be an important factor in the progression of AD pathogenesis, but first need to identify the underlying AD-related mechanisms (Jiang et al., 2019).

Other media, such as saliva, have been proposed to provide diagnostic information for AD. A total of 6,230 metabolites from saliva were tested, and three were found to differentiate between MCI, AD, and cognitively normal patients (Huan et al., 2018).

None of these tests or biomarkers are valid as a stand-alone diagnostic test. The lack of standardized techniques makes diagnostic accuracy across all scenarios difficult to achieve. Current AD diagnostic standards using evaluation of clinical presentation have maintained a high level of accuracy, combined with the lack of a clinical treatment make all early AD diagnostic tests and biomarkers of limited clinical utility (François et al., 2019; Schaffer et al., 2015). However, research criteria have incorporated both molecular and topographic biomarker data into the research definitions of both symptomatic and pre-symptomatic forms of AD, anticipating that once biomarkers become more standardized, they will be incorporated into clinical diagnostic algorithms for AD (Morris et al., 2014; Wolk & Dickerson, 2024).

Proprietary Testing

Proprietary tests exist for assessment of AD biomarkers. C_2N Diagnostics offers PrecivityAD™, a blood test that measures the ratio of Aß42 to Aß40 and ApoE detection. C_2N Diagnostics received a "Breakthrough Device Designation" from the FDA in January 2019 for their test measuring the ratio of Aß42 to Aß40 (C2N, 2019). Fujirebio Diagnostics offers the *in vitro* Lumipulse® G β-Amyloid Ratio (1-42/1-40) test, which combines the results of the Lumipulse® G β-Amyloid 1-42 and Lumipulse® G β-Amyloid 1-40 to create a ratio of beta-amyloid 1-42 and beta-amyloid 1-40 concentrations in CSF with the LUMIPULSE G1200 system (Fujirebio, 2022). This is intended to predict the likelihood of amyloid plaque formation in potential AD. This assay received the "Breakthrough Device Designation" from the FDA in May 2022 (FDA, 2022).

Roche Diagnostics received 501(k) clearance from the FDA in 2022 for their Elecsys® beta-Amyloid (1-42) CSF II (Abeta42) and Elecsys® Phospho-Tau (181P) CSF (pTau181) assays in 2022 for adults 55 years and older who are evaluated for the disease and other cognitive impairments to generate a pTau181/Abeta42 ratio value. The company cites that these assays "achieve 90% concordance with the Amyloid PET scan imaging and have the potential to provide a more affordable and accessible routine option to confirm the presence of amyloid in the brain." They can also detect pathology in earlier stages of disease due to the correlative changes in biomarkers (Roche, 2022). In June 2023, Roche Diagnostics

also received 501(k) clearance from the FDA for the Elecsys® beta-Amyloid (1-42) CSF II (Abeta42) and Elecsys® Total-Tau CSF assays (tTau) in the same population through the tTau/Abeta42 ratio, and will be available in Q4 2023. The company endorses that these assays provide a cost-effective, more widely available alternative to the recommended PET imaging option with minimal radiation exposure. The ratio would be "consistent with a negative amyloid PET scan if the result is less than or equal to the cutoff (negative), and with a positive amyloid PET scan if the result is above the ratio cutoff (positive) (Roche, 2023).

On July 6, 2023, Quanterix® launched the LucentAD test, which measures serum levels of tau protein phosphorylated at Thr181 (p-Tau 181), which is a marker of AD pathology. It is intended to assist in the diagnostic evaluation of AD with other tools, but clues providers into a patient's likelihood of amyloid-related pathology. It is not currently approved by the FDA, but has been studied in conjunction with the drug lecanemab in its effectiveness for treating AD therapy response (BusinessWire, 2023).

Lecanemab is an antibody intravenous (IV) infusion therapy. It works by targeting and removing beta-amyloid from the brain. It has received traditional approval from the U.S. Food and Drug Administration (FDA) to "treat early Alzheimer's disease, including people living with mild cognitive impairment (MCI) or mild dementia due to Alzheimer's disease who have confirmation of elevated beta-amyloid in the brain" (Alzheimer's Association, 2024). Lecanemab lowers beta-amyloid in the brain, reducing cognitive and functional decline in people living with early Alzheimer's. This treatment is appropriate for people with early Alzheimer's who have been confirmed to have elevated beta-amyloid levels. The test is appropriate in those with elevated beta-amyloid levels and as such, before treatment could be considered, the physician would first need to confirm the presence of beta-amyloid plaques. Currently, the FDA does not specify a diagnostic tool to determine elevated beta-amyloid. However, some examples of tools to diagnose beta-amyloid elevation include PET scan or lumbar puncture (CSF) tests (Alzheimer's Association, 2024).

The Syn-One Test offered by CND Life Sciences is intended to aid in the diagnosis of synucleinopathies, which includes Parkinson Disease (PD), dementia with Lewy bodies, multiple system atrophy, and pure autonomic failure. Using a skin biopsy, the test detects (through immunostaining) abnormal (phosphorylated) alpha-synuclein (P-Syn) in skin nerve fibers. The Syn-One pathology report also includes results for small fiber neuropathy and amyloidosis (CND Life Sciences).

There is a growing body of evidence confirming the association of P-Syn with various synucleinopathies and P-Syn's potential utility as a diagnostic biomarker. Donadio et al. (2019) reported "excellent" inter- and intra-laboratory reproducibility when using the skin biopsy technique to assay for P-Syn, providing increased confidence that this approach may be feasible across institutions. Levine et al. (2021) showed that P-Syn might be useful as a marker for other disease states (postural orthostatic tachycardia syndrome), expanding the potential applications of the analyte for different clinical scenarios.

Despite the growing support, other studies have highlighted some concerns: Kim et al. (2019) reviewed the available data regarding the use of cutaneous alpha-synuclein as a marker for synucleinopathies and confirmed that a high specificity is consistently reported across studies, but that sensitivity measurements can vary widely. The authors suggest that this is likely influenced by the specific type of disease, as well as differences in design and methodology (i.e., biopsy site, tissue thickness, or fixation methods). Waqar et al. (2023) published a similar review that emphasized the variability in reported sensitivity of P-Syn detection from skin biopsies, as well as the small sample sizes in many of the published studies to date. The authors do acknowledge, however, the many advantages of using skin samples, including the low cost and high patient tolerance of the technique.

Clinical Utility and Validity

Dage et al. (2016) studied the correlation of tau protein levels (in plasma) with neuronal damage. A total of 378 cognitively normal (CN) patients were examined, along with 161 patients with mild cognitive impairment (MCI). Baseline plasma tau protein levels were measured. The authors found that plasma tau levels were higher in MCI patients compared to CN patients (4.34 pg/mL for MCI compared to 41.4 pg/ML for CN, p = .078). The authors also performed a regression accounting for age, gender, education, and ApoE, which suggested that higher plasma tau levels were associated with worse memory loss and abnormal cortical thickness (Dage et al., 2016).

Lewczuk et al. (2017) compared the ratio of Aβ42/40 to just Aβ42 as measurements of clinical AD. A total of 200 patients (150 PET-negative, 50 PET-positive for amyloid) were examined and compared to the positron emission tomography (PET) results. The authors found that the ratio of Aβ42/40 agreed more strongly with the PET results (89.4% concordance compared to 74.9% concordance for Aβ42 only). A larger area under the curve was found for the Aβ42/40 measurement compared to just Aβ42 (0.936 compared to 0.814). The authors concluded that "the CSF Aβ42/40 ratio is superior to Aβ42 alone as a marker of amyloid-positivity by PET" (Lewczuk et al., 2017).

Talwar et al. (2016) performed a meta-analysis on CSF ApoE levels in AD patients. Twenty-four studies, including 1064 AD cases and 1338 healthy controls, were reviewed. The authors found that although the total sample did not indicate a significant association between AD and ApoE levels, a subgroup analysis controlling for sample size (n > 43) indicated significantly lower ApoE levels in AD patients compared to controls. The authors considered CSF ApoE levels to have "potential" as an indicator of AD association (Talwar et al., 2016).

Wang et al. (2018) evaluated the clinical value of α-synuclein in MCI and AD. The investigators added α-synuclein and phosphorylated α-synuclein to a biomarker panel containing Aβ42, tau, and phosphorylated tau and evaluated the new panel's performance. A total of 729 CSF samples were taken. The phosphorylated version of α-synuclein was found to weakly associate with diagnosis at baseline, but total α-synuclein was not. CSF α-synuclein was found to predict the Alzheimer Disease Assessment Scale-Cognitive, memory, executive function, and progression from MCI to AD. Longitudinal biomarker changes were not found to differ between groups. Overall, α-synuclein was found to potentially better predict AD changes better than the classic biomarkers (Wang et al., 2018).

Zhang et al. (2014) performed a meta-analysis focusing on urinary Alzheimer-associated neuronal thread protein (AD7c-NTP)'s diagnostic ability for AD. Nine studies were reviewed for probable and possible AD, and the authors evaluated AD7c-NTP's sensitivity at 0.87, specificity at 0.89, positive likelihood ratio at 8.13, and negative likelihood ratio at 0.15 (Zhang et al., 2014).

Wang et al. (2019) explored the potential of urinary extracellular vesicle (EV) biomarkers in neurological disorders, including AD, Parkinson Disease (PD), and Huntington Disease (HD). A discovery cohort of 50 individuals was used to create the initial set of EV proteins and a set of 108 individuals was used to further develop the list of biomarkers. The authors identified "hundreds" of commonly expressed EV proteins with stable expression. SNAP23 and calbindin were most elevated in PD cases, with an 86% prediction of diagnostic success in the discovery cohort and 76% prediction of diagnostic success in the replication cohort. Moreover, "Broad Gene set analysis (GSEA) further reveals a prominent link to Alzheimer disease with 10.4% of the genes known to be down-regulated in the brains from patients with Alzheimer disease identified in urinary EVs" (Wang et al., 2019).

Liu et al. (2018) examined the urinary metabolic profile of β-amyloid 25-35 (Aβ 25-35)-injected rats. This was intended to establish AD in the rats, allowing the impairment of spatial learning and memory to be tested in the rats after eight weeks. The authors identified the characteristic AD symptoms after eight weeks (cognitive dysfunction, hippocampus damage, Aβ formation and tau phosphorylation) as well as 45 altered metabolites involving eight metabolic pathways. The investigators concluded that "pathogenesis of AD was mainly due to gut microbiome dysbiosis, inhibition of energy metabolism, oxidative stress injury and loss of neuronal protective substances" (Liu et al., 2018).

Fossati et al. (2019) studied the correlation of plasma tau with cerebrospinal fluid (CSF) tau and phosphorylated tau (P-tau). A total of 97 subjects were included (68 healthy controls and 29 AD patients). Plasma tau was found to be higher in AD patients compared to healthy controls (area under curve: 0.79). However, CSF tau and plasma tau were "poorly" correlated. The addition of plasma tau to the receiver operating curve of CSF tau increased the area under curve to 0.82 from 0.80 and increased the curve of P-tau to 0.88 from 0.87. The authors concluded that "adding plasma tau to CSF tau or P-tau improves diagnostic accuracy, suggesting that plasma tau may represent a useful biomarker for AD" (Fossati et al., 2019).

Tatebe et al. (2017) developed an immunoassay to quantify plasma p-tau181. Three cohorts were used to validate the assay. In the first cohort (20 AD patients, 15 controls), the tau levels were found to be higher in the AD patients (0.171 ± 0.166 pg/ml in AD versus 0.0405 ± 0.0756 pg/ml in controls). In the second cohort (20 Down Syndrome patients, 22 controls), the tau levels were higher in the Down Syndrome patients (0.767 ± 1.26 pg/ml in DS versus 0.0415 ± 0.0710 pg/ml in controls). Finally, in the third cohort (eight AD patients, three other neurological diseases), the tau levels were found to correlate well with the CSF tau levels (r^2 = 0.4525). Overall, the authors suggested that "that the plasma p-tau181 is a promising blood biomarker for brain AD pathology" (Tatebe et al., 2017).

Shen et al. (2019) completed a meta-analysis review of 170 studies to research the role of inflammatory markers in AD and MCI. Increased periphery levels, compared to controls, were found with many types of biomarkers including high-sensitivity C reactive protein, $p<0.05$; interleukin-6, $p<0.005$; soluble tumour necrosis factor receptor 1, $p<0.005$; soluble tumour necrosis factor receptor 2, $p<0.005$; alpha1-antichymotrypsin, $p<0.005$; IL-1β, $p<0.05$; soluble CD40 ligand, $p<0.05$; CSF levels of IL-10, $p<0.05$; monocyte chemoattractant protein-1, $p<0.005$; transforming growth factor-beta 1, $p<0.05$; soluble triggering receptor expressed on myeloid cells2, $p<0.001$; YKL-40, $p<0.001$; α1-ACT, $p<0.001$; nerve growth factor, $p<0.005$; and visinin-like protein-1, $p<0.005$ (Shen et al., 2019). The authors conclude that all the significant relationships found in this large meta-analysis help to support "the notion that AD and MCI are accompanied by inflammatory responses in both the periphery and CSF" (Shen et al., 2019).

Palmqvist et al. (2019) analyzed two different, cross-sectional, multicenter studies (n=1079). The CSF Aβ42/Aβ40 ratio was used to identify AD via Elecsys immunoassays from Roche Diagnostics; further, plasma neurofilament light chain (NFL), heavy chain (NFH), and *APOE* genotype were also analyzed in the first cohort of patients (n=842). "In cohort 1, plasma Aβ42 and Aβ40 predicted Aβ status with an area under the receiver operating characteristic curve (AUC) of 0.80 (95% CI, 0.77-0.83). When adding *APOE*, the AUC increased significantly to 0.85 (95% CI, 0.82-0.88)" (Palmqvist et al., 2019). Cohort 2 had similar results with a slightly higher AUC (0.86; 95% CI, 0.81-0.91). The authors conclude by stating that "Plasma Aβ42 and Aβ40 measured using Elecsys immunoassays predict Aβ status in all stages of AD with similar accuracy in a validation cohort. Their accuracy can be further increased by analyzing *APOE* genotype" (Palmqvist et al., 2019).

Kim et al. (2020) studied the diagnostic utility of multiplexed sensing to detect multiple AD biomarkers (t-tau, p-tau181, Aβ42, and Aβ40) in human plasma using densely aligned carbon nanotubes (CNT). The CNT sensor assay exhibited superior sensitivity and precision, enabling the platform to accurately quantify AD biomarkers despite the hundreds of other agents in the blood plasma. The densely aligned CNT sensor array was 10–10^3 times more sensitive than the commercially available sandwich-type or enzyme-linked immunosorbent assay. The authors conclude that "by measuring the levels of t-tau/Aβ42, p-tau181/Aβ42, and Aβ42/Aβ40 in clinical blood samples, the sensor array successfully discriminates the clinically diagnosed AD patients from healthy controls with an average sensitivity of 90.0%, a selectivity of 90.0%, and an average accuracy of 88.6%" (Kim et al., 2020).

Simrén et al. (2021) studied the diagnostic and prognostic potential of plasma biomarkers in Alzheimer disease. Various biomarkers, including phosphorylated-tau181 (P-tau181), neurofilament light, amyloid-β (Aβ42/40), total-tau and glial fibrillary acidic protein, were analyzed in 99 cognitively unimpaired (CU) patients, 107 mild cognitive impairment (MCI) patients, and 103 Alzheimer disease (AD) patients. According to the results, P-tau181 significantly outperformed all biomarkers in differentiating AD dementia from CU. Higher P-tau181 value was associated with increased cognitive decline and gray matter loss in temporal regions. The authors conclude that "these findings highlight the potential value of plasma P-tau181 as a non-invasive and cost-effective diagnostic and prognostic biomarker in AD" (Simrén et al., 2021).

Qu et al. (2021) performed a systematic review and meta-analysis of 150 studies aiming to evaluate the effect of AD biomarkers on blood. The authors performed a "random-effects meta-analysis based on the ratio of means method and multivariable-adjusted effect estimates." The results demonstrated that T-tau, P-tau and Nfl increased, and that AβPPR decreased from controls to amnestic MCI (aMIC) to AD. Aβ42, Aβ42/40, and P-tau217 all had valid diagnostic accuracy. The authors conclude that the significant changes in core blood biomarkers support that "biomarkers were strongly valid in identifying AD" (Qu et al., 2021).

Chen et al. (2021) performed a meta-analysis of 17 studies aimed at calculating the diagnostic accuracy of blood-based biomarkers. The authors compared the diagnostic odds ratio (DOR) of biomarkers between controls, AD, and aMCI conditions. When comparing AD and control groups, the plasma Aβ42 DOR was 32.2 (sensitivity = 88 %, specificity = 81 %), the plasma Aβ oligomer DOR was 29.1 (sensitivity = 80 %, specificity = 88 %), and the plasma tau DOR was 52.1 (sensitivity = 90 %, specificity = 87 %). When comparing aMCI and controls, the plasma Aβ42 DOR was 60.4 (sensitivity = 86 %, specificity = 90 %), and the plasma tau DOR was 49.1 (sensitivity = 79 %, specificity = 94 %). The authors conclude that blood-based biomarkers are "minimally invasive and cost-effective tools for detecting AD; however, the evidence for detecting aMCI was still limited" (Chen et al., 2021).

Yoong et al. (2021) performed a systematic review and meta-analysis of 13 studies aiming to address the prognostic utility of a new CSF biomarker: Neurogranin (Ng). Core CSF biomarkers such as Aβ42, T-tau, and P-tau can support AD diagnosis, but cannot predict AD progression. Ng has been shown to predict cognitive decline. The authors found evidence that CSF Ng can predict Mini-Mental State Examination (MMSE) decline in $Aβ^+$ MCI patients and the decline of memory and executive function in MCI. Additionally, CSF Ng/Aβ42 was also found likely to predict cognitive decline. The authors conclude that CSF Ng may be an applicable AD biomarker, but more studies are required to validate 548309its use (Yoong et al., 2021).

Nojima et al. (2022) investigated the clinical utility of measuring CSF biomarkers through the LUMIPULSE® system in correlation with Aβ deposition status confirmed by amyloid PET. From 199 CSF

samples from patients with confirmed AD and underwent amyloid PET, measurements of Aβ 1–40 (Aβ40), Aβ 1–42 (Aβ42), total tau (t-Tau), and phosphorylated tau-181 (p-Tau181) using the LUMIPULSE system were taken and analyzed with a multivariable logistic regression model. Through this, they were able to determine that there was diagnostic agreement between the biomarker levels and amyloid PET imaging, and that there was statistical significance in the association between amyloid PET status and Aβ40 and Aβ42, with the ratios providing better diagnostic agreement than single biomarkers alone. Researchers also determined that the statistically significant correlation between the Aβ42/Aβ40 ratio and p-Tau181 may render a plausible utility in predicting brain Aβ pathology. The CSF findings may also potentially draw parallels to benefits with measuring blood plasma levels of the AD biomarkers with high-sensitivity assays, but the "plasma biomarker levels could be affected by small measurement variations caused by preanalytical handling and analytical performance, leading to misclassification (i.e., false-negative or false-positive for Aβ pathology)" (Nojima et al., 2022).

Guidelines and Recommendations

National Institute of Neurological and Communicative Disorders and Stroke (NINCDS) and the Alzheimer's Disease and Related Disorders Association (ADRDA)

In 1984, the NINCDS and ADRDA developed clinical criteria for the diagnosis of AD. While evidence to date has used NINCDS/ADRDA's AD classification, in 2011, the National Institute on Aging and the Alzheimer's Association workgroup revised diagnostic criteria for diagnosis of dementia due to Alzheimer's disease (McKhann et al., 2011).

The biomarkers reviewed in this policy are included in a category among revisions to AD diagnostic criteria- "probable AD dementia with evidence of the AD pathophysiological process". However, the diagnostic criteria workgroup publication noted "we do not advocate the use of AD biomarker tests for routine diagnostic purposes at the present time. There are several reasons for this limitation: 1) the core clinical criteria provide very good diagnostic accuracy and utility in most patients; 2) more research needs to be done to ensure that criteria that include the use of biomarkers have been appropriately designed, 3) there is limited standardization of biomarkers from one locale to another, and 4) access to biomarkers is limited to varying degrees in community settings. Presently, the use of biomarkers to enhance certainty of AD pathophysiological process may be useful in three circumstances: investigational studies, clinical trials, and as optional clinical tools for use where available and when deemed appropriate by the clinician" (McKhann et al., 2011).

Alzheimer's Association

The Alzheimer's Association has initiated a quality control program for CSF markers, noting that "Measurements of CSF AD biomarkers show large between laboratory variability, likely caused by factors related to analytical procedures and the analytical kits. Standardization of laboratory procedures and efforts by kit vendors to increase kit performance might lower variability, and will likely increase the usefulness of CSF AD biomarkers" (Mattsson et al., 2011).

In 2013, the Alzheimer's Association published recommendations for operationalizing the detection of cognitive impairment in the primary care setting (Cordell et al., 2013). It stated that "the use of biomarkers (e.g., CSF tau and beta amyloid proteins, amyloid tracer positron emission tomography scans) was not considered as these measures are not currently approved or widely available for clinical use."

In 2018, a multidisciplinary group of the Alzheimer's Association published criteria for lumbar puncture and CSF testing in the diagnosis of AD. The committee recommends CSF biomarker testing for six clinical indications deemed appropriate, as listed in the table below.

Table 1: Clinical indications for appropriate use of LP and cerebrospinal fluid testing in the diagnosis of AD (Shaw et al., 2018)

No.	Indication	Ratings
1	Cognitively unimpaired and within normal range functioning for age as established by objective testing; no conditions suggesting high risk and no SCD [subjective cognitive decline] or expressed concern about developing AD	Inappropriate
2	Cognitively unimpaired patient based on objective testing, but considered by patient, family informant, and/or clinician to be at risk for AD based on family history	Inappropriate
3	Patients with SCD (cognitively unimpaired based on objective testing) who are considered to be at increased risk for AD	**Appropriate**
4	Patients with SCD (cognitively unimpaired based on objective testing) who are not considered to be at increased risk for AD	Inappropriate
5	MCI that is persistent, progressing, and unexplained	**Appropriate**
6	Patients with symptoms that suggest possible AD	**Appropriate**
7	MCI or dementia with an onset at an early age (<65)	**Appropriate**
8	Meeting core clinical criteria for probable AD with typical age of onset	**Appropriate**
9	Symptoms of REM sleep behavior disorder	Inappropriate
10	Patients whose dominant symptom is a change in behavior (e.g., Capgras Syndrome, paranoid delusions, unexplained delirium, combative symptoms, and depression) and where AD diagnosis is being considered	**Appropriate**
11	Use to determine disease severity in patients having already received a diagnosis of AD	Inappropriate
12	Individuals who are apolipoprotein E (APOE) ε4 carriers with no cognitive impairment	Inappropriate
13	Use of LP in lieu of genotyping for suspected ADAD mutation carriers	Inappropriate
14	ADAD mutation carriers, with or without symptoms	Inappropriate

Abbreviations: AD, Alzheimer's disease; LP, lumbar puncture; REM, rapid eye movement; SCD, subjective cognitive decline; ADAD, autosomal dominant Alzheimer's disease; MCI, mild cognitive impairment.

The workgroup has also identified several gray areas where more research is needed. The authors note that "One question that will need further data is whether measuring a ratio of CSF Aβ42/40 yields better diagnostic performance than measuring Aβ42 alone. Another question is how to characterize neurodegeneration using CSF biomarkers, and whether neurodegeneration in the absence of positive amyloid biomarkers predicts progression in persons with MCI" (Shaw et al., 2018). Further, the authors

also state that "much more work is needed to document the potential impact of CSF AD biomarker testing on clinical outcomes in patients across the spectrum of AD" (Shaw et al., 2018).

In 2022, the Alzheimer's Association published a recommendation on the appropriate use of blood-based biomarkers (BBMs) in AD. In this recommendation, they note that BBMs are showing promise in revolutionizing the diagnostic and prognostic work-up of AD. However, they still caution that BBMs should not yet be used as primary endpoints in pivotal trials. The recommend that BBMS may be cautiously used in specialized memory clinics, but additional data are needed before BBMS can be used as a stand-alone diagnostic AD marker or before they should be considered for use in primary care. While they do recognize that BBMs are useful as (pre)-screeners for clinical trials, they only cautiously recommend the use of BBMs outside the clinical trial setting:

"Recommendations of the use of AD-associated BBMs in clinical trials and practice:

Use of BBMs in specialized memory clinic settings:

(5) BBMs (with established thresholds) should currently only be used in symptomatic patients at specialist clinics and the results should be confirmed whenever possible with CSF or PET. Additional data are needed before use of BBMs as stand-alone diagnostic markers.

Use of BBMs in primary care:

(6) Additional data are needed for use of BBMs in primary care" (Hansson et al., 2022).

"That said, the implementation of such markers in trials and practice must be done in a careful and controlled way not to accidentally cause more harm than good. Much more research is therefore needed before widespread clinical use of BBMs as we have outlined above. Such research is also needed before the community can establish Appropriate Use Criteria for clinical use of BBMs, which is a prerequisite for general use of such markers in the clinic. However, the acquired experience from implementation of CSF AD biomarkers and Aβ-PET in many countries will ensure rapid validation of relevant BBMs in the first contexts of use, including trials and specialized memory clinics. The implementation of BBMs in primary care will likely take much longer, because relevant and high-quality research studies on AD-related BBMs in this setting are very few, but hopefully more prospective studies will be launched in the coming years using relevant and accurate reference standards" (Hansson et al., 2022)

Expert Working Group for the EU Joint Program—Neurodegenerative Disease Research (JPND) BIOMARKAPD Program

An expert working group, comprised of 28 international members, was convened to develop recommendations for CSF AD biomarkers in the diagnostic evaluation of dementia. "The working group recommended using the CSF biomarkers in MCI as an add-on to clinical evaluation alone for predicting functional decline or progression to AD dementia and, based on the available evidence, the recommendation was strong. However, in comparison with the outcome of using hippocampal atrophy as a biomarker, the working group issued a weak recommendation to incorporate CSF biomarkers in the diagnostic workup compared with hippocampal atrophy. Because of insufficient evidence, the working group could not recommend CSF biomarkers as an alternative to FDG-PET or amyloid-PET in predicting future decline or conversion. The working group recommended using CSF biomarkers to inform future

disease management, but the strength of this recommendation was weak because of the small amount of evidence" (Simonsen et al., 2017).

Six clinical questions were asked by Simonsen et al. (2017):

1. "In patients with MCI, will CSF biomarkers (alone or in combination) compared with (A) clinical measures alone and/or (B) other imaging biomarkers identify or exclude AD as the cause of MCI?"
    a. Final recommendation: N/A
2. "In patients with MCI, will CSF biomarkers (alone or in combination) compared with (A) clinical measures alone and/or (B) other imaging biomarkers predict conversion to AD dementia within 3 years?"
    a. Final recommendation: Yes, strong
3. "In patients with MCI, will CSF biomarkers (alone or in combination) compared with (A) clinical measures alone and/or (B) other imaging biomarkers predict functional or cognitive decline?"
    a. Final recommendation: Yes, strong
4. "In patients with MCI, will CSF biomarkers (alone or in combination) compared with (A) clinical measures alone and/or (B) other imaging biomarkers change disease management?"
    a. Yes, weak
5. "In patients with MCI, will CSF biomarkers (alone or in combination) compared with (A) clinical measures alone and/or (B) other imaging biomarkers improve patient well-being?"
    a. Yes, weak
6. "In patients with MCI, will CSF biomarkers (alone or in combination) compared with (A) clinical measures alone and/or (B) other imaging biomarkers reduce health care costs?"
    a. No, weak

Additional recommendations were made by Herukka et al. (2017) for CSF AD biomarkers in the diagnostic evaluation of mild cognitive impairment. The same six clinical questions were asked as above by Simonsen et al. (2017):

1. "In patients with MCI, will CSF biomarkers (alone or in combination) compared with (A) clinical measures alone and/or (B) other imaging biomarkers identify or exclude AD as the cause of MCI?"
    a. Final recommendation: N/A
2. "In patients with MCI, will CSF biomarkers (alone or in combination) compared with (A) clinical measures alone and/or (B) other imaging biomarkers predict conversion to AD dementia within 3 years?"
    a. Final recommendation: Yes, strong
3. "In patients with MCI, will CSF biomarkers (alone or in combination) compared with (A) clinical measures alone and/or (B) other imaging biomarkers predict functional or cognitive decline?"
    a. Final recommendation: Yes, strong
4. "In patients with MCI, will CSF biomarkers (alone or in combination) compared with (A) clinical measures alone and/or (B) other imaging biomarkers change disease management?"
    a. Yes, weak
5. "In patients with MCI, will CSF biomarkers (alone or in combination) compared with (A) clinical measures alone and/or (B) other imaging biomarkers improve patient well-being?"
    a. Yes, weak
6. "In patients with MCI, will CSF biomarkers (alone or in combination) compared with (A) clinical measures alone and/or (B) other imaging biomarkers reduce health care costs?"
    a. No, weak

National Institute on Aging (NIA, NIH) and Alzheimer's Association

In 2011, the National Institute on Aging and Alzheimer's Association workgroups published guidelines for the diagnosis of AD. The authors also note that "Two fundamental issues about individuals with MCI may be answered by the use of biomarkers: (1) To establish support for the underlying etiology of the clinical syndrome in an individual with MCI, which will have major importance for choosing the correct therapy, when effective treatments are available. (C2N) To determine the likelihood of cognitive and functional progression for an individual MCI patient to a more severe stage of MCI or to dementia, and the likelihood that this progression will occur within a defined period" (Albert et al., 2011). The authors also note that "in these recommendations, CSF tau is considered to be a strong marker of the neuronal injury associated with AD. However, the two biomarkers in combination are extremely informative. Together with low CSF Aβ42, elevated CSF tau provides a high likelihood of progression to AD in patients with MCI;" however, because many biochemical events may be associated with AD, the authors confirm that "Additional work in this area is needed to know how useful these markers will be" (Albert et al., 2011).

In 2018, guidelines were published by the National Institute on Aging and Alzheimer's Association for the preclinical, mild cognitive impairment, and dementia stages of AD, and are intended for use in observational and interventional research, not routine clinical care. These guidelines state that "there is now a growing consensus that application of biomarkers should be harmonized conceptually across the disease continuum and that biomarkers of neurodegeneration are not equivalent to those reflecting amyloid and pathologic tau accumulation" (Jack et al., 2018). Further, regarding the guidelines noted above from 2011, the authors state that "Studies published since 2011 have reinforced the idea that certain imaging and CSF biomarkers are valid proxies for neuropathologic changes of AD…. additional research has highlighted the fact that measures of neurodegeneration or neuronal injury that are commonly used in AD research—magnetic resonance imaging (MRI), fluoro-deoxyglucose (FDG) PET, and CSF total tau (T-tau)—are not specific for AD but rather are nonspecific indicators of damage that may derive from a variety of etiologies, for example, cerebrovascular injury" (Jack et al., 2018). The authors also state that the "data firmly establish that more advanced disease defined by biomarkers predicts greater likelihood of and more rapid cognitive decline. Thus, a solid evidence base exists proving that combinations of biomarker abnormalities are useful for staging the Alzheimer's continuum" (Jack et al., 2018).

Global Biomarker Standardization Consortium (GBSC)

The GBSC of the Alzheimer's Association has noted that before biomarkers can be used in clinical practice, they "must be standardized and validated on a global scale" (GBSC, 2024).

American Academy of Neurology (AAN)

In 2018, a guideline was issued as an update to the 2001 AAN guideline on mild cognitive impairment (MCI) and endorsed by the Alzheimer's Association. This guideline was reaffirmed in 2021 (AAN, 2021). The panel determined that the field of biomarkers is rapidly evolving. And, according to the panel, there are no biomarkers that that could clearly predict progression in patients with MCI. They have provided the following recommendations:

> Recommendation A7a
> "For patients and families asking about biomarkers in MCI, clinicians should counsel that there are no accepted biomarkers available at this time (Level B)."

Recommendation A7b
"For interested patients, clinicians may discuss the option of biomarker research or refer patients or both, if feasible, to centers or organizations that can connect patients to this research (e.g., subspecialty centers, Trial Match, ClinicalTrials.gov) (Level C)."

In 2001, the Quality Standards Committee of the American Academy of Neurology issued a "Practice parameter: Diagnosis of dementia (an evidence-based review)." Relevant statements to the current policy include the following:

"...no laboratory tests have yet emerged that are appropriate or routine use in the clinical evaluation of patients with suspected AD. Several promising avenues genotyping, imaging and biomarkers are being pursued, but proof that a laboratory test has value is arduous. Ultimately, the putative diagnostic test must be administered to a representative sample of patients with dementia who eventually have pathologic confirmation of their diagnoses. A valuable test will be one that increases diagnostic accuracy over and above a competent clinical diagnosis."

"There are no CSF or other biomarkers recommended for routine use in determining the diagnosis of AD at this time" (Knopman et al., 2001).

Dementia with Lewy Bodies (DLB) Consortium

The DLB Consortium published a consensus report on the diagnosis and management of dementia with Lewy bodies, which are characteristic of Alzheimer's Disease and other neurological conditions. The Consortium states that "direct biomarker evidence of LB-related pathology is not yet available for clinical diagnosis" (McKeith et al., 2017).

Consensus of the Task Force on Biological Markers in Psychiatry of the World Federation of Societies of Biological Psychiatry

The Federation published an update on cerebrospinal fluid (CSF) and blood biomarkers for neurodegenerative dementias. The Federation considers blood-based biomarkers to "offer an ideal complementary step to advanced CSF and neuroimaging biomarkers and can serve as the first-step in a multi-stage process", although these biomarkers still require validation and "a great deal of additional work" (Lewczuk et al., 2018).

International Working Group (IWG)

Dubois et al. (2014) published a position paper which presents a new diagnostic algorithm for AD which states: "Aβ1–42 and tau (T-tau or P-tau) should be used in combination, and the CSF AD signature, which combines low Aβ1 and high T-tau or P-tau concentrations, significantly increases the accuracy of AD diagnosis even at a prodromal stage. This combination reaches a sensitivity of 90–95% and a specificity of about 90% in AD. CSF biomarkers cannot be used as standalone tests and should be interpreted in a larger clinical context with confounding factors considered. An important concern is the large variability in CSF measures between laboratories and across techniques, and the lack of agreement on cutoff thresholds. These variations have made direct comparison of study results difficult. Several programmes of standardisation, including the Alzheimer's Association Quality Control programme for CSF biomarkers, initiatives within the Joint Program for Neurodegenerative Diseases, and the Global Biomarker Standardisation Consortium, and by industry, will minimise between-laboratory variations in the future and allow identification of uniform cutoff levels." In their 2021 IWG position paper, the group states "Overall, evidence for the use of biomarkers in clinical practice remains highly disputed and

suffers from a dearth of evidence-based data to recommend biomarker assessments for cognitively unimpaired individuals." (Dubois et al., 2021).

The IWG describes specific biochemical evidence in their definitions of AD:

"In-vivo evidence of Alzheimer's pathology (one of the following):

- Decreased Aβ1–42 together with increased T-tau or P-tau in CSF
- Increased tracer retention on amyloid PET
- AD autosomal dominant mutation present (in PSEN1, PSEN2, or APP)" (Dubois et al., 2014).

In their updated proposed recommendations, the IWG included the following relevant information:

1. "The diagnosis of Alzheimer's disease is clinical–biological. It requires the presence of both a specific clinical phenotype of Alzheimer's disease (phenotype positive) and biomarker evidence of Alzheimer's disease pathology (amyloid-positive and tau positive).
2. In people who have…common phenotypes, amyloid and tau biomarker positivity establishes an Alzheimer's disease diagnosis (table 2). The positivity of both amyloid and tau biomarkers is required because an amnestic phenotype with only amyloid positivity is not specific to Alzheimer's disease and is seen in other neurodegenerative diseases with amyloid copathology (including LATE and dementia with Lewy bodies) or in patients with cerebral amyloid angiopathy and amnestic vascular cognitive impairment. However, an isolated amnestic syndrome of the hippocampal type with only tau biomarker positivity can occur in primary age-related tauopathy or in atypical presentations of mixed 3 repeat or 4 repeat tau frontotemporal lobar degeneration. Finally, uncommon phenotypes with positive Alzheimer's disease biomarkers should not be a-priori classified as an established Alzheimer's disease (table 2); in such cases the clinician could deem that Alzheimer's disease is not the dominant pathology driving the clinical phenotype but only a copathology.
3. Recommended biomarker measures for amyloid β pathology are low CSF Aβ42, increased CSF Aβ40–Aβ42 ratio (which is, if possible, preferred to low CSF Aβ42) or high tracer retention in amyloid PET. For tau pathology, we recommend high CSF phosphorylated tau (not total tau because of low specificity) or increased ligand retention in tau PET. Recommendation of amyloid PET and tau PET for use in clinical practice is conditional on regulatory approval and reimbursement by payers in different countries.
4. CSF investigation is prioritized because it provides simultaneous information on the two types of biomarkers (amyloid β and tau) and is less expensive than amyloid PET, tau PET, or both. If lumbar puncture is contraindicated, PET investigations are an alternative.
5. In clinical practice, plasma biomarkers for amyloid β and tau pathology are not currently recommended. Although promising, plasma biomarkers require further standardization and validation before they can be broadly regarded as secure evidence of Alzheimer's disease pathology (amyloid-positive and tau-positive).
6. In clinical practice, the investigation of pathophysiological biomarkers in cognitively unimpaired individuals is not recommended, given the current inability to predict reliable clinical trajectories of people who are asymptomatic with biomarker positive status (amyloid-positive and tau-positive). In the future, if therapies or prevention programmes show substantial efficacy in delaying onset of disease, that will probably change the need for biomarker investigations in these individuals, although the problem of the prediction of clinical trajectories in cognitively unimpaired biomarker-positive individuals will still remain.

7. Physicians are recommended to evaluate the added-value of biomarker investigation for each symptomatic patient objectively, according to the clinical situation (age, risk of comorbidity, complexity of the phenotype), the life context, the wishes of the patient to know the most likely diagnosis, the possibility of participation in a disease-modifying trial, and the appreciation of how this information will change the management of the patient. Biomarker investigations can also be limited by the availability, cost, and health-care payment coverage of biomarkers across countries, centres, and clinical situations" (Dubois et al., 2021).

Table 2 (Dubois et al., 2021):

	Likelihood of Alzheimer's disease as a primary diagnosis	Further investigation
Common Alzheimer's disease phenotypes (amnestic variant, logopenic variant of primary progressive aphasia, and posterior cortical atrophy)		
Amyloid positive, tau positive	Highly probable–established	None required
Amyloid positive, tau unknown	Probable	Consider a tau measure (PET, CSF)
Amyloid positive, tau negative	Probable	Consider an additional tau measure (PET, CSF)
Tau positive, amyloid unknown	Possible	Consider an amyloid measure (PET, CSF)
Tau positive, amyloid negative	Possible	Consider an additional amyloid measure (PET, CSF)
Amyloid negative, tau unknown	Unlikely	Full investigation of cause and consider a tau measure (PET, CSF)*
Amyloid unknown, tau negative	Unlikely	Full investigation of cause and consider an amyloid measure (PET, CSF)*
Amyloid negative, tau negative	Highly unlikely–excluded	Full investigation of cause*‡
Amyloid unknown, tau unknown	Non-assessable	Consider tau and amyloid measures (PET, CSF)
Uncommon Alzheimer's disease phenotypes (behavioural or dysexecutive variant, corticobasal syndrome, non-fluent variant of primary progressive aphasia, and semantic variant of primary progressive aphasia)		
Amyloid positive, tau positive	Probable	None required; careful follow-up needed: an incongruent clinical phenotype and neurodegeneration pattern should trigger a new investigation*
Amyloid positive, tau unknown	Possible	Consider a tau measure (PET, CSF)
Amyloid positive, tau negative	Possible	Consider an additional tau measure (PET, CSF)
Tau positive, amyloid unknown	Unlikely	Full investigation of cause and consider an amyloid measure (PET, CSF)
Tau positive, amyloid negative	Unlikely	Full investigation of cause*
Amyloid negative, tau unknown	Highly unlikely–excluded	Full investigation of cause*‡
Amyloid negative, tau negative	Highly unlikely–excluded	Full investigation of cause*‡
Amyloid unknown, tau negative	Highly unlikely–excluded	Full investigation of cause*‡
Amyloid unknown, tau unknown	Non-assessable	Full investigation of cause and consider tau and amyloid measures (PET, CSF)*
Other phenotypes (eg, dementia with Lewy bodies, Richardson syndrome, Huntington's disease, and amyotrophic lateral sclerosis)		
Amyloid positive, or tau positive, or both	Unlikely	Full investigation of cause*
Amyloid negative, tau unknown	Highly unlikely–excluded	Full investigation of cause*
Amyloid unknown, tau negative	Highly unlikely–excluded	Full investigation of cause*
Amyloid negative, tau negative	Highly unlikely–excluded	Full investigation of cause*
Amyloid unknown, tau unknown	Highly unlikely–excluded	Full investigation of cause*

United States Preventive Services Task Force (USPSTF)

In 2020, the USPSTF published a recommendation stating that "current evidence is insufficient to assess the balance of benefits and harms of screening for cognitive impairment in older adults" (Owens et al., 2020).

European Federation of Neurological Societies (EFNS)

The EFNS published updated guidelines in 2012 for the diagnosis and management of disorders associated with dementia. These guidelines state that "Routine CSF analysis may help to rule out or rule in certain infectious causes (Good Practice Point). CSF abeta 1-42/tau/p-tau assessment helps to differentiate AD (Level B). Assessment of CSF total tau and 14-3-3 protein is recommended in rapidly progressive dementia when sCJD is suspected (Good Practice Point)" (Sorbi et al., 2012).

Canadian Consensus Conference on the Diagnosis and Treatment of Dementia (CCCDTD)

In 2020, the CCCDTD released recommendations on the diagnosis and treatment of dementia. The guidelines state that "CSF analysis is not recommended routinely, but it can be considered in dementia patients with diagnostic uncertainty and onset at an early age (<65) to rule out Alzheimer's disease (AD) pathophysiology." The guidelines also state that "CSF analysis can also be considered in dementia patients with diagnostic uncertainty and predominance of language, visuospatial, dysexecutive, or behavioral features to rule out AD pathophysiology" (Ismail et al., 2020).

National Institute for Health and Care Excellence (NICE)

In 2018, NICE released guidelines on assessment, management, and support for people living with dementia. With regards to further testing, NICE states to only consider them if "it would help diagnosed a dementia subtype and knowing more about the dementia subtype would change management."

The NICE recommendations for further tests for Alzheimer's disease are delineated below:

- "If the diagnosis is uncertain… and Alzheimer's disease is suspected, consider either:
    - FDG-PET (fluorodeoxyglucose-positron emission tomography-CT), or perfusion SPECT (single-photon emission CT) if FDG-PET is unavailable **OR**
    - Examining cerebrospinal fluid for:
        - Either total tau or total tau and phosphorylated-tau 181 **and**
        - Either amyloid beta 1-42 or amyloid beta 1-42 and amyloid beta 1-40.
    - If a diagnosis cannot be made after one of these tests, consider using the other one.
- Be aware that the older a person is, the more likely they are to get a false positive with cerebrospinal fluid examination.
- Do not rule out Alzheimer's disease based solely on the results of CT or MRI scans.
- Do not use Apolipoprotein E genotyping or electroencephalography to diagnose Alzheimer's disease.
- Be aware that young-onset Alzheimer's disease has a genetic cause in some people" (NICE, 2018).

Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

On February 15, 2018, the FDA released a statement concerning the advancement of the development of novel treatments for neurological conditions, including Alzheimer disease. FDA Commissioner Scott Gottlieb, M.D., states, "Symptoms and progression of neurological diseases can also vary significantly across patients, and even within patients, and across organ systems. Some diseases, like Alzheimer's, may progress invisibly for years. Once clinical symptoms become apparent, significant function may already be lost. These issues can make drug development more challenging for companies and are deeply frustrating for patients and caregivers living with these serious and life-threatening conditions. The FDA recognizes the urgent need for new medical treatments for many serious conditions including neurological disorders such as muscular dystrophies, amyotrophic lateral sclerosis (ALS), Alzheimer's disease (AD), migraine and epilepsy. This requires us to become more nimble, collaborative and patient-focused. As part of our ongoing efforts to expand access to safe and effective treatment options across all disease areas and promote innovation, the FDA is modernizing multiple aspects of our drug regulatory programs – including how we communicate scientific and regulatory guidance for drug development" (Gottlieb, 2018). Concurrently, the FDA released a guidance for industry concerning AD for public comment for 90 days. Within the guidance, the FDA states, "FDA supports and endorses the use of diagnostic criteria that are based on a contemporary understanding of the pathophysiology and evaluation of AD… Important findings applicable to the categorization of AD along its continuum of progression include the presence of pathophysiological changes as measured by biomarkers, the presence or absence of detectable abnormalities on sensitive neuropsychological measures, and the presence or absence of functional impairment manifested as meaningful daily life impact the present with subjective complaints or reliable observer reports" (FDA, 2024). The final draft of the guidance should be released in the future after the public comment period has concluded.

In 2022, the FDA permitted marketing for the Fujirebio Diagnostics Lumipulse® G β-Amyloid Ratio (1-42/1-40) test, which is administrated under a CMS laboratory certification process. It is intended to measure the ratio of beta-amyloid 1-42 and beta-amyloid 1-40 concentrations in CSF, which can help predict the likelihood of amyloid plaque formation in potential AD. This assay received the "Breakthrough Device Designation" from the FDA in May 2022 (FDA, 2022).

Roche Diagnostics received 501(k) clearance from the FDA for their Elecsys® beta-Amyloid (1-42) CSF II (Abeta42) and Elecsys® Phospho-Tau (181P) CSF (pTau181) assays in 2022 for adults 55 years and older who are evaluated for the disease to generate a pTau181/Abeta42 ratio value. In June 2023, Roche Diagnostics also received 501(k) clearance from the FDA for the Elecsys® beta-Amyloid (1-42) CSF II (Abeta42) and Elecsys® Total-Tau CSF assays (tTau) in the same population through the tTau/Abeta42 ratio, and will be available in Q4 2023 (Roche, 2023).

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
83520	Immunoassay for analyte other than infectious agent antibody or infectious agent antigen; quantitative, not otherwise specified

0206U	Neurology (Alzheimer disease); cell aggregation using morphometric imaging and protein kinase C-epsilon (PKCe) concentration in response to amylospheroid treatment by ELISA, cultured skin fibroblasts, each reported as positive or negative for Alzheimer disease Proprietary test: DISCERN™ Lab/Manufacturer: NeuroDiagnostics
0207U	Quantitative imaging of phosphorylated ERK1 and ERK2 in response to bradykinin treatment by in situ immunofluorescence, using cultured skin fibroblasts, reported as a probability index for Alzheimer disease (List separately in addition to code for primary procedure) Proprietary test: DISCERN™ Lab/Manufacturer: NeuroDiagnostics
0289U	Neurology (Alzheimer disease), mRNA, gene expression profiling by RNA sequencing of 24 genes, whole blood, algorithm reported as predictive risk score Proprietary test: MindX Blood Test™ - Memory/Alzheimer's Lab/Manufacturer: MindX Sciences™ Laboratory/MindX Sciences™ Inc
0346U	Beta amyloid, Aβ40 and Aβ42 by liquid chromatography with tandem mass spectrometry (LC-MS/MS), ratio, plasma Proprietary test: QUEST AD-Detect™, Beta-Amyloid 42/40 Ratio, Plasma Lab/Manufacturer: Quest Diagnostics
0358U	Neurology (mild cognitive impairment), analysis of β-amyloid 1-42 and 1-40, chemiluminescence enzyme immunoassay, cerebral spinal fluid, reported as positive, likely positive, or negative Proprietary test: Lumipulse® G β-Amyloid Ratio (1-42/1-40) Test Lab/Manufacturer: Fujirebio Diagnostics, Inc
0393U	Neurology (eg, Parkinson disease, dementia with Lewy bodies), cerebrospinal fluid (CSF), detection of misfolded α-synuclein protein by seed amplification assay, qualitative Proprietary test: SYNTap® Biomarker Test Lab/Manufacturer: Amprion Clinical Laboratory
0412U	Beta amyloid, Aβ42/40 ratio, immunoprecipitation with quantitation by liquid chromatography with tandem mass spectrometry (LC-MS/MS) and qualitative ApoE isoform-specific proteotyping, plasma combined with age, algorithm reported as presence or absence of brain amyloid pathology Proprietary test: PrecivityAD® blood test Lab/Manufacturer: C2N Diagnostics LLC
0443U	Neurofilament light chain (nfl), ultra-sensitive immunoassay, serum or cerebrospinal fluid Proprietary test: Neurofilament Light Chain (NfL) Lab/Manufacturer: Neuromuscular Clinical Laboratory at Washington University in St. Louis School of Medicine, Neuromuscular Clinical Laboratory at Washington University in St. Louis School of Medicine
0445U	B-amyloid (abeta42) and phospho tau (181p) (ptau181), electrochemiluminescent immunoassay (eclia), cerebral spinal fluid, ratio reported as positive or negative for amyloid pathology Proprietary test: Elecsys® PhosphoTau (181P) CSF (pTau181) and βAmyloid (1-42) CSF II (Abeta 42) Ratio Lab/Manufacturer: Roche Diagnostics Operations, Inc (US owner/operator)
0459U	β-amyloid (Abeta42) and total tau (tTau), electrochemiluminescent immunoassay (ECLIA), cerebral spinal fluid, ratio reported as positive or negative for amyloid pathology Proprietary test: Elecsys® Total Tau CSF (tTau) and βAmyloid (1-42) CSF II (Abeta 42) Ratio

	Lab/Manufacturer: Roche Diagnostics Operations, Inc (US owner/operator)
0479U	Tau, phosphorylated, pTau217 Proprietary test: ALZpath pTau217 Lab/Manufacturer: Neurocode USA, Inc, Quanterix/ALZpath
0503U	Neurology (Alzheimer disease), beta amyloid (Aβ40, Aβ42, Aβ42/40 ratio) and tau-protein (ptau217, np-tau217, ptau217/nptau217 ratio), blood, immunoprecipitation with quantitation by liquid chromatography with tandem mass spectrometry (LC-MS/MS), algorithm score reported as likelihood of positive or negative for amyloid plaques Proprietary test: PrecivityAD2™ Lab/Manufacturer: C2N Diagnostics, LLC

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

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Revision History

Revision Date	Summary of Changes
09/04/2024	Reviewed and Updated: Updated background, guidelines, and evidence-based scientific references. Literature review necessitated the following changes in coverage criteria: New CC1: "1) For individuals with Alzheimer disease or mild cognitive impairment, measurement of amyloid beta peptides in cerebrospinal fluid MEETS COVERAGE CRITERIA." Results in edit to former CC1, now CC2, remove amyloid beta peptides from the list of unallowed CSF biomarkers. Now reads: "2) Measurement of cerebrospinal fluid biomarkers of Alzheimer disease or dementia not mentioned above (e.g., tau protein, α-synuclein, or neural thread proteins) DOES NOT MEET COVERAGE CRITERIA." Added CPT code 0479U, 0503U (effective date 10/1/2024).

06/19/2024	Off-cycle coding modification: Added CPT code 0459U (effective date 7/1/2024)
03/06/2024	Off-cycle coding modification: Added CPT code 0443U, 0445U (effective date 4/1/2024)

Biomarker Testing for Autoimmune Rheumatic Disease

Policy Number: AHS – G2022 – Biomarker Testing for Autoimmune Rheumatic Disease	Prior Policy Name and Number, as applicable: AHS-G2022-ANA/ENA Testing
Initial Presentation Date: 11/16/2015 Revision date: 03/06/2024	

Policy Description

Systemic autoimmune rheumatic diseases (SARDs) are a diverse group of conditions that primarily affect the joints, bones, muscle, and connective tissue (AAFP, 2019). SARDs are characterized by dysregulated immunity and inflammatory responses, resulting in damage and destruction to joints, connective tissues, skin, blood elements, and other target organs; however, considerable diversity in clinical presentation, disease course, and treatment response exists (Guthridge et al., 2022).

The diagnostic workup for SARDs may involve the antinuclear antibody (ANA) assay, which is used to detect autoantibodies (AAB) against intracellular antigens, originally known as antinuclear antibodies (Tan, 1989). Commonly used as part of the initial diagnostic workup to screen for evidence of systemic autoimmunity (Satoh et al., 2014), detection and identification of AABs are important in the diagnosis of SARDs, such as systemic lupus erythematosus (SLE), Sjögren's syndrome (SjS), mixed connective tissue disease (MCTD), systemic sclerosis (SSc), and idiopathic inflammatory myopathies (IIMs) (Tebo, 2017). Extractable nuclear antigens or ENAs

(a historical term from when the antigens were extracted from the cell into saline solution prior to testing) include Sm, U1 ribonucleoprotein (RNP), Ro, and La antigens, and are also useful for evaluating individuals with suspected connective tissue disease (Bloch, 2022).

Related Policies

Policy Number	Policy Title
AHS-G2155	General Inflammation Testing

Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) For individuals with a clinical suspicion of autoimmune disease, testing for antinuclear antibodies (ANA) **MEETS COVERAGE CRITERIA**:
   a) Once per lifetime in individuals with stable symptoms.
   b) Repeat testing only if a significant change in symptoms occurs.

2) For individuals with an abnormal, raised ANA titer or with abnormal immunological findings in the serum and a clinical correlation with the appropriate autoimmune disorder, extractable nuclear antigens (ENA) panel testing of specific autoantibodies **MEETS COVERAGE CRITERIA**.

3) For individuals with painful and swollen joints and a clinical suspicion of rheumatoid arthritis, testing for rheumatoid factor (RF) and/or anti-cyclic citrullinated peptide (anti-CCP) antibodies **MEETS COVERAGE CRITERIA**:
   a) Once per lifetime in individuals with stable symptoms.
   b) Repeat testing only if a significant change in symptoms occurs.

4) For individuals with an initial positive ANA test and a diagnosis of systemic autoimmune rheumatic disease, testing of dsDNA up to four (4) times per year **MEETS COVERAGE CRITERIA**.

5) For individuals with a negative or low positive ANA test, the following condition specific antibody testing **MEETS COVERAGE CRITERIA**:
   a) Testing for anti-Jo-1 in a unique clinical subset of myositis.
   b) Testing for anti-SSA in the setting of lupus or Sjögren's syndrome.

6) Monitoring of disease with ANA testing or ANA titers **DOES NOT MEET COVERAGE CRITERIA**.

7) For individuals without symptoms suggestive of an autoimmune disorder, ANA and/or ENA testing **DOES NOT MEET COVERAGE CRITERIA**.

8) For all other situations not described above, testing of specific antibodies in the absence of a positive ANA test **DOES NOT MEET COVERAGE CRITERIA**.

9) For asymptomatic individuals, testing of ANA and/or ENA during a wellness visit or a general exam without abnormal findings **DOES NOT MEET COVERAGE CRITERIA**.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

10) For the management of rheumatoid arthritis (RA), serum biomarker panel testing (e.g., Vectra DA score, PrismRA) **DOES NOT MEET COVERAGE CRITERIA**.

11) For the diagnosis of systemic lupus erythematosus (SLE), the use of cell-bound complement activation products (e.g., AVISE Lupus) **DOES NOT MEET COVERAGE CRITERIA**.

12) For the diagnosis, prognosis, or monitoring of SLE or connective tissue diseases, serum biomarker panel testing with proprietary algorithms and/or index scores (e.g., AVISE CTD, AVISE SLE Monitor, AVISE SLE Prognostic) **DOES NOT MEET COVERAGE CRITERIA**.

Table of Terminology

Term	Definition
AAB	Autoantibodies
AAP	American Academy of Pediatrics
ACL	Anticardiolipin
ACP	American College of Pathologists
ACPA	Anti-citrullinated peptide antibodies
ACR	American College of Rheumatology
AIH	Autoimmune hepatitis
AIIF	Automated indirect immunofluorescence
ANA	Antinuclear antibody
Anti La/SS-B	Anti La/Sjogren Syndrome-B
Anti-C1q	Autoantibodies against C1q
Anti-CCP	Anti-cyclic citrullinated peptide
Anti-dsDNA	Anti-double-stranded DNA
Anti-RNP	Antinuclear ribonucleoprotein
Anti-Ro/SS-	Anti-Ro/Sjogren Syndrome related antigen A autoantibodies
Anti-Sm	Anti-Smith antibodies
APL	Antiphospholipid antibodies
BC4d	B-lymphocyte-bound C4d
BSR	British Society for Rheumatology
CBC	Complete blood count

CB-CAPs	Cell-bound complement activation products
CCP	Cyclic citrullinated peptides
CDC	Centers for Disease Control and Prevention
CENP	Centromere protein B
CIA	Chemiluminescence immunoassay
CLIA ’88	Clinical Laboratory Improvement Amendments of 1988
CMS	Centers for Medicare and Medicaid Services
CRP	C-reactive protein
CTD	Connective tissue diseases
CV	Coefficient of variation
ds	Double-stranded
dsDNA	Double-stranded DNA
EC4d	C4d bound to erythrocytes
eGFR	Estimated glomerular filtration rate
EIA	Enzyme immunoassay
ELISA	Enzyme-linked immunosorbent assay
ENA	Extractable nuclear antigens
ESPGHAN	European Society for Paediatric Gastroenterology Hepatology and
ESR	Erythrocyte sedimentation rate
EULAR	European League Against Rheumatism
FEIA	Fluorescence enzyme immunoassay
HEp-2	Human epithelial type 2
ICAP	International Consensus on ANA staining Patterns
IFA	Immunofluorescence assay
IIF	Indirect immunofluorescence
IIMs	Idiopathic inflammatory myopathies
IQ	Interquartile
ISLM	Italian Society of Laboratory Medicine
JIA	Juvenile idiopathic arthritis
Jo-1	Histidyl t-RNA synthetase
LAC	Lupus anticoagulant
LDT	Laboratory developed test
LE cell	Lupus erythematosus cell
LFA	Lupus Foundation of America
MAP	Multianalyte assay panel
MCTD	Mixed connective tissue disease
MIA	Multiplex immunoassay
MIIF	Manual indirect immunofluorescence
PC	Positive concordance
PMPM	Per member per month
PPPM	Per patient per month
RA	Rheumatoid arthritis
RF	Rheumatoid factor
RNP	Ribonucleoprotein
SARDs	Systemic autoimmune rheumatic diseases

SDI	SLICC damage index
SDLT	Standard diagnosis laboratory testing
SELENA	Safety of Estrogens in Lupus National Assessment
SjS	Sjögren's syndrome
SLE	Systemic lupus erythematosus
SLICC	Systemic Lupus International Collaborating Clinics
SRDs	Systemic rheumatic diseases
SS-B/La	Sjogren's syndrome Type-B
SSc	Systemic sclerosis

Scientific Background

Autoimmune diseases occur when an individual's immune system mistakenly attacks his or her own tissue. This can lead to a variety of conditions and diseases which vary in severity. Autoimmune diseases are estimated to affect 5% of the world population with estimated to affect 8% of the US population (Global Autoimmune Institute, 2022); autoimmune conditions are associated with increased morbidity and mortality, and are among the leading causes of death (under 65 years) and disability for women in the US (Simon et al., 2017).

Systemic lupus erythematosus (SLE) is one of more than 80 known autoimmune disorders, affecting approximately 23.2/100,000 people in the United States (Rees et al., 2017). The Lupus Foundation in America recently reported that lupus affects approximately 1.5 million people in the United States (LFA, 2021). SLE can present with a wide range of clinical manifestations, typically related to connective-tissue disorders, and often mimics other illnesses (Zucchi et al., 2019). This autoimmune disorder leads to inflammation and irreversible damage in one or more organs, including the joints, skin, nervous system, and kidneys (Durcan et al., 2019). The cause of SLE is not entirely understood, but it is predicted to manifest due to a combination of genetic and environmental factors, such as vitamin D deficiency, sunburn, and/or viral infections (Finzel et al., 2018). SLE affects women more than men and is a challenging disease to diagnose because of a broad assortment of signs, symptoms, and serological abnormalities (Durcan et al., 2019). SLE morbidity can be attributed to both tissue damage, toxic treatments, and complications associated with treatments, such as immunosuppression, long-term organ damage due to corticosteroid therapy, and accelerated coronary artery disease (Durcan et al., 2019; Fava & Petri, 2019). An early SLE diagnosis is particularly challenging as early-stage tests lack specificity; further, clinical signs and symptoms often only appear after organ damage has occurred, indicating later stages of the disease (Thong & Olsen, 2017). SLE diagnoses are made based on lab findings, clinical manifestations, serology, and histology of impacted organs (Thong & Olsen, 2017). However, current SLE screening tests are notoriously unreliable (Bhana, 2023).

Rheumatoid arthritis (RA) affects more than one million adults in the United States. RA is characterized by chronic inflammation of the synovial tissue of joints, cartilage, and bone (Cohen et al., 2021; Johnson et al., 2019; Luan et al., 2021; Pappas et al., 2021; Scherer et al., 2020). Pathological abnormalities in patients with RA includes chronic synovitis, which results in joint devastation (Johnson et al., 2019; Luan et al., 2021; Scherer et al., 2020). Cellular and

humoral response aberrations result in autoimmunity; antibodies and rheumatoid factors against post-translational modified proteins (including modifications such as citrullination). As such, synthetic cyclic citrullinated peptides (CCP) have been developed for diagnostic use (Scherer et al., 2020).

There is consensus to the value of serological testing for diagnostic purposes: both rheumatoid factor (RF) and anti-citrullinated peptide antibodies (ACPA) tests have diagnostic value in patients suspected of having RA (but not in asymptomatic patients as a general screen) (Baker, 2023). Diagnostic testing with RF should be restricted to those with a moderate to high pretest probability of rheumatoid arthritis. RF testing should not occur in patients with joint pain in the absence of synovitis (e.g., nonspecific arthralgias, fibromyalgia, OA) because a positive test result is more likely to represent a false-positive result. ACPA testing is useful as a diagnostic test in patients with a moderate to high pretest probability of rheumatoid arthritis, but similarly, should not be used in those with a low pre-test probability. For patients "with an inflammatory, small joint arthritis and with a moderate to high pretest probability of RA, the presence of ACPA testing confirms a diagnosis of RA" (Baker, 2023).

To date, the etiology of RA has not been fully elucidated, though recent studies have suggested that genetic, epigenetic, and environmental factors contribute to RA presentation (Johnson et al., 2019; Scherer et al., 2020). Due to the complexity of RA pathogenesis, there is no model drug to cure RA.

Biologic markers or "biomarkers" can provide objective measurements that reflect underlying pathophysiological processes, pathogenic processes, or responses to treatment. Most measures of monitoring disease and treatment progress rely on subjective measurements, such as joint evaluation, so biomarkers may be a useful complement in patient management (Taylor & Maini, 2022). Joint damage at the molecular level may be occurring before any clinical signs appear so identifying any indications of disease activity could allow clinical interventions to be taken earlier(McArdle et al., 2015). Markers such as erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) are part of clinical measures such as the DAS. However, these two biomarkers are nonspecific; abnormal amounts of these markers may be due to other reasons apart from RA and may be completely normal in patients with RA (Centola et al., 2013; Curtis et al., 2012). This non-specificity is not limited to ESR and CRP. For example, antibodies (usually called rheumatoid factors or RF) produced against immunoglobulin G (IgG) are often tested to diagnose RA, but these antibodies may be produced in response to another rheumatic condition or a separate chronic infection (Shmerling, 2023). Autoantibodies to citrullinated protein epitopes, such anti-cyclic citrullinated peptide (anti-CCP2), has also been a focus of biomarker research in RA. Both RF and anti-CCP2 have similar sensitivities for the diagnosis of RA, but anti-CCP2 is positive in 20%-30% of RA patients who are negative for RF (Shapiro, 2021). RA is a heterogenous condition, and no single biomarker is a reliable predictor of RA disease activity (McArdle et al., 2015).

Currently, conventional synthetic disease modifying anti-rheumatic drugs (csDMARDs) are the first line of RA therapy. Unfortunately, some RA patients do not respond to csDMARDs and clinical guidelines suggest use of alternative therapies such as biologic DMARDs (bDMARDs).

bDMARDs are more specific to inflammatory factors than csDMARDs and more efficient in demonstrating remission and inducing low disease activity (Castro et al., 2022). Several bDMARDs are available for RA management, and these include TNFis (Bergman et al., 2020; Johnson et al., 2019; Luan et al., 2021; Pappas et al., 2021). TNFi treatment, however, is not without limitations. Unfortunately, the majority of patients fail to respond to TNFi treatment (measured by American College of Rheumatology (ACR)50-indicates 50% disease improvement) and only 10-25% achieve remission (Cohen et al., 2021; Curtis et al., 2014; Johnson et al., 2019; Pappas et al., 2021). Currently, there is no way to predict whether RA patients will respond to TNFi therapy, and approximately three months is needed to determine whether a patient is responding (Johnson et al., 2019; Pappas et al., 2021). Accordingly, there has been a push to create a personalized medicine approach to identify non-responders to enhance clinical outcomes (Johnson et al., 2019; Pappas et al., 2021).

The systems by which the immune system maintains tolerance to an individual's own antigens can be overcome by release of intracellular antigens following excessive cell death, ineffective clearance of apoptotic debris, inflammation-induced modification of self-antigens, or molecular mimicry, leading to the production of antibodies against self-antigens or autoantibodies (AAB) (Suurmond & Diamond, 2015). Autoantibodies mediate both systemic inflammation and tissue injury and may play a role in the pathogenesis of many autoimmune diseases (Suurmond & Diamond, 2015). Generally, AAB development precedes the clinical onset of autoimmune disease (Damoiseaux et al., 2015) and has predictive value (Satoh et al., 2014); thus, AABs serve as good serological markers to screen for evidence of autoimmunity (Aggarwal, 2014). Autoantibodies can target a variety of molecules (including nucleic acids, lipids, and proteins) from many cellular localizations—nucleus, cytoplasm, cell surface, extracellular organelles (Suurmond & Diamond, 2015), and different specific AABs are associated with particular diagnoses, symptoms, unique syndromes, subsets of disease, and clinical activity (Satoh et al., 2014). See Table 1 from Suurmond and Diamond (2015), below:

Table 1. Autoantibody recognition in systemic autoimmune disease

Antigen location	Antibody	Antigen	Disease	PRR recognition
Nuclear	Anti-Ro (SS-a)	Ro-RNP complex	SLE, Sjögren's syndrome	TLR7
	Anti-La (SS-B)	La antigen	SLE, Sjögren's syndrome	TLR7
	Anti-Sm	Small nuclear RNP	SLE	TLR7
	Anti-dsDNA	dsDNA	SLE	TLR9
	Anti-histone	Histones	SLE (drug-induced)	TLR2 and TLR4
	Anti–Scl-70	Topoisomerase I	Systemic sclerosis	
	Anti-centromere	Centromere	Systemic sclerosis/CREST syndrome	
Cytoplasmic/ mitochondrial	ANCA	Myeloperoxidase (p-ANCA) and proteinase 3 (c-ANCA)	Vasculitis, Wegener's granulomatosis	
	ACA	Cardiolipin	Antiphospholipid syndrome, SLE	NLRP3
Modified proteins	ACPA	Citrullinated proteins	RA	TLR4
	Anti-Carp	Carbamylated proteins	RA	
Extracellular	RF	RF (IgG)	RA	
	Lupus anticoagulant	Phospholipids	Antiphospholipid syndrome	TLR4?
	α3 Chain of basement membrane collagen (type IV collagen)	α3 Chain of basement membrane collagen (type IV collagen)	Goodpasture's syndrome	

ACA, anti-cardiolipin antibody.

However, serum AAB are present in 18.1% of the general population, and titers are higher in females and increase with age (Selmi et al., 2016). Additionally, only in a few cases does the

antibody titer correlates with the severity of clinical manifestations or the response to treatment (Damoiseaux et al., 2015). The use of ANA detection as a diagnostic test originated with the observation of the lupus erythematosus (LE) cell (Hargraves et al., 1948). Since then, several tests have been developed to detect these antibodies.

The indirect immunofluorescence (IIF) test is the most widely used assay for the detection of AAB and remains the reference method of choice (ACR, 2015). Detection of ANAs by the IIF technique demonstrates binding to specific intracellular structures within the cells, resulting in staining patterns reported using the consensus nomenclature and representative patterns defined by The International Consensus on ANA staining Patterns (ICAP) initiative (Chan et al., 2016) and the degree of binding reflected by the fluorescence intensity or titer (Tebo, 2017). The test takes advantage of a HEp-2 cell line, which have large, easy to visualize, nuclei and contain nearly all of the clinically important autoantigens, making these cells ideal for the detection of the corresponding AABs (Bloch, 2023). The ANA IIF assay using HEp-2 slide has a high sensitivity for screening of SARDs and efforts to harmonize the nomenclatures for testing and reporting (Chan et al., 2015) have made this a powerful screening tool (Tebo, 2017). The frequency of ANA in SLE and SSc is 95–100%, 50–70% in SJS and 30–50% in rheumatoid arthritis (RA) (Satoh et al., 2014); however, their isolated finding in an otherwise healthy individual has a low positive predictive value which needs to be integrated with other laboratory parameters and patient risk factors (Selmi et al., 2016). Disadvantages of the indirect immunofluorescence test include its labor-intensiveness, significant training requirements for competence, and subjectivity in titer and pattern recognition; moreover, because the staining pattern usually does not identify the responsible autoantibody, additional testing may be required (Bloch, 2023; Tebo, 2017). Automated image analysis provides a viable option for distinguishing between positive and negative results although the ability to assign specific patterns is insufficient to replace manual microscopic interpretation (Yoo et al., 2017).

The antinuclear antibody (ANA) test is commonly used in the evaluation of autoimmune disorders, as these antibodies are responsible for attacking healthy or normal cells. More than 95% of individuals with SLE will have a positive ANA test (Bhana, 2023). However, ANAs are present in "a significant proportion of normal individuals and lacks specificity or prognostic value" (Thong & Olsen, 2017). In particular, approximately only 11-13% of individuals with a positive ANA test will actually have SLE, and approximately 15% will be completely healthy (Bhana, 2023). Other SLE diagnostic methods include the monitoring of anti-double-stranded DNA (anti-dsDNA), C3 and C4 complement levels, CH50 complement levels, erythrocyte sedimentation rate (ESR) and/or C-reactive protein (CRP) levels, antiphospholipid antibodies, and urine protein-to-creatinine ratios (Wallace & Gladman, 2023).

If SLE is suspected based on the clinical picture following a positive ANA screen, the sera should be tested for antibodies to double-stranded DNA (dsDNA). Anti-dsDNA antibodies are present in two-thirds of patients with SLE, and they have a good association with disease activity and lupus nephritis. Serial monitoring of anti-dsDNA antibodies has modest correlation with disease activity (Aggarwal, 2014).

A positive ANA screen should also be followed by identification of sub-specificities by screening for antibodies to extractable nuclear antigens (ENAs). ENAs were identified by using saline extract of nuclei as the antigen. Antibodies to ENA can be determined using double immunodiffusion, immunoblotting, enzyme-linked immunosorbent assays (ELISA), or bead-based assay using recombinant or affinity-purified antigens. Different ENAs have an association with different connective tissue diseases (Aggarwal, 2014).

Reflex tests for positive ANA screens have been proposed to improve appropriateness in diagnosis of SARDs and avoid unnecessary second level testing. For specific autoantibodies responsible for certain fluorescent ANA patterns, such as homogeneous, speckled, fine grainy (Scl70-like), nucleolar, centromeric or speckled cytoplasmic, the identification of precise autoantibody markers is considered essential while for others it is not deemed to be necessary (Tonutti et al., 2016). See Table 1 from Tonutti et al, 2016, below.

Table 1

ANA-reflex test procedure with titres ≥1:160 and typical patterns

ANA-IIF pattern on HEp-2 cells	Reflex test(s)
Nuclear homogeneous ≥1:160	Antibodies to intracellular specific antigens (ENA) and to dsDNA/nucleosomes
Nuclear speckled ≥1:160	Anti-dsDNA and antibodies to intracellular specific antigens (ENA), possibly including anti-RNA polymerase III
Nuclear Scl70-like ≥1:160	Antibodies to intracellular specific antigens (ENA) (possibly including anti-PM/Scl)
Cytoplasmic speckled ≥1:160	Antibodies to intracellular specific antigens (ENA), including anti-tRNA synthetases and anti-P ribosomal
Pleomorphic PCNA-like (any titre)	Anti-PCNA
Centromere	No confirmation necessary if high titres. Execute specific test for anti-CENP B only in dubious cases (low titre or centromeric pattern not clearly recognizable)

ENA includes SS-A/Ro52 and Ro60, SS-B/La, Sm, RNP, Jo-1, and Scl70

Proprietary Testing

A set of proprietary tests are available from Exagen, under the “AVISE” line. Their line of tests utilizes a two-tiered testing method and a novel algorithm that measures 10 SLE relevant markers to deliver an index calculation value suggestive of the presence or absence of SLE. This includes tests for prognosis (10 biomarkers including various autoantibodies such as anti-C1q and antiribosomal P), diagnosis (10 biomarkers, includes ENA panel), and monitoring (6 biomarkers, includes anti-dsDNA and anti-C1q). AVISE CTD (standing for connective tissue disease) is intended to assist with the differential diagnosis of several autoimmune diseases and includes several ANA biomarkers, as well as an ENA panel. Other tests offered, such as AVISE Anti-CarP (evaluates autoantibodies to carbamylated proteins for rheumatoid patients) still include ANA components (AVISE, 2024).

AVISE Lupus by Exagen is a laboratory developed test (LDT) designed to assist in SLE diagnoses. This LDT utilizes a two-tiered testing method and a novel algorithm that measures 10 SLE relevant markers to deliver an index calculation value suggestive of the presence or absence of SLE. The AVISE Lupus test also uses cell-bound complement activation products (CB-CAPs) to measure complement system activation (Exagen, 2024). The 10 SLE relevant markers in this test include anti-dsDNA, anti-Smith (anti-Sm) antibodies, erythrocyte-bound C4d or B-lymphocyte-bound C4d (BC4d), ANA, CB-CAPs, and autoantibody specificity

components (Exagen, 2024). As noted on their website, "The AVISE Lupus test is an ideal test for ANA positive patients with a clinical suspicion of lupus" (Exagen, 2024).

PrismRA is a molecular signature test that predicts TNFi non-response prior to treatment initiation. PrismRA utilizes a 23-feature blood-based molecular signature response classifier (MSRC) which integrates next generation RNA sequencing data and clinical features (clinical metrics, demographic variables, C reactive protein (CRP) and anti-CCP antibodies) to predict patients' response to TNFi treatment (Cohen et al., 2021). A high score is indicative of decreased likelihood of the RA patient to respond to TNFi therapies.

Vectra DA is a multi-biomarker disease activity (MBDA) blood test which combines the levels of 12 serum biomarkers into a single score from 1 to 100 to provide an objective measure of RA disease activity. It is intended for use with existing symptom-based disease activity measures to improve long-term outcomes for RA patients (van der Helm-van Mil et al., 2013). While multi-biomarker panels are emerging as a potentially useful tool in the management of RA, there is not yet a consensus as to their clinical utility (Taylor & Maini, 2022).

Analytical Validity

A variety of manual or automated single or multiplex immunoassays have been introduced to make the process of detecting autoantibodies more efficient, including ELISA, fluorescent microsphere assays, and chemiluminescence immunoassays (CIA)—each with different performance characteristics (Tebo, 2017). In these assays, a panel of purified native or recombinant autoantigens is prepared, and each antigen is immobilized on a solid surface (microtiter plate, fluorescent microsphere, or membrane) and incubated with diluted human serum (Bloch, 2023). The advantages of these alternative approaches to ANA IIF testing include their suitability for high-throughput testing, semi-quantification of test results, the lack of subjectivity, and the consolidation of ANA-related tests in a single platform as a positive test also provides identification of the responsible autoantibody (Bloch, 2023; Tebo, 2017). It has been estimated that solid phase assays may decrease the labor cost of ANA testing by as much as 95 percent (Bloch, 2023). In a recent study which evaluated the performance of an automated CIA and fluorescence enzyme immunoassay (FEIA) and compared their performance to that of IIFA, both FEIA and CIA screen significantly outperformed IIF, with a higher specificity for FEIA and higher sensitivity for CIA (van der Pol et al., 2018). The use of solid phase assays as the initial test for the detection of ANA is concerning because the number of autoantigens that are included in solid phase assays is limited compared with the number that are present in the Hep-2 cell substrate, thus limiting sensitivity (Bloch, 2023). Consequently, IIF remains the gold standard, and in cases of strong clinical suspicion of SARD and a negative screen from a solid phase assay, IIF should be performed (van der Pol et al., 2018).

Tipu and Bashir (2018) investigated the specificity and pattern for ANA in systemic rheumatic disease patients. 4347 samples were sent, and 397 were positive for ANA. Of these 397, 96 were positive on the anti-ENA screen and tested for anti-ENA reactivity. Anti-SSA antibodies were found in 59 of these samples. The most common ANA patterns were "coarse" and "fine-

speckled" (43 and 22 of 81 respectively). However, no specific ANA pattern was associated with anti-ENA reactivity (Tipu & Bashir, 2018).

Kim et al. (2019) performed a meta-analysis comparing ANA measurement by automated indirect immunofluorescence (AIIF) and manual indirect immunofluorescence (MIIF). 22 studies including 6913 positive and 1818 negative samples of manual indirect immunofluorescence (MIIF) were included. Among this cohort, 524 samples with combined systemic rheumatic diseases (SRDs), 132 systemic lupus erythematosus (SLE) samples, and 104 systemic sclerosis (SSc) samples, and 520 controls were available. Positive concordance (PC) between AIIF and MIIF was 93.7%, although PC of total pattern and titer were lower. Clinical sensitivities of AIIF vs MIIF were 84.7% vs 78.2% for combined SRDs, 95.5% vs 93.9% for SLE, and 86.5% vs 83.7% for SSc. Clinical specificities of AIIF vs MIIF were 75.6% vs 79.6% for combined SRDs, 74.2% vs 83.3% for SLE, and 74.2% vs 83.3% for SSc. The authors concluded that the sensitivities did not differ between methods, but the specificities of SLE and SSc were statistically significant changes (Kim et al., 2019).

Dervieux et al. (2017) performed the analytical validation of Exagen's multianalyte panel test for SLE. This assay uses quantitative flow cytometry to assess the levels of the complement split product C4d bound to erythrocytes (EC4d) and B-lymphocytes (BC4d), in units of mean fluorescence intensity (MFI), and immunoassays to assay for antinuclear and anti-double stranded DNA antibodies (e.g. autoantibodies). The results were reported on a two-tiered index score as either positive or negative. The authors included specimens from both patients with SLE as well as individuals without SLE. Controls consisting of three-level C4 coated positive beads were run daily. The authors note that at ambient temperature both EC4d and BC4d are stable for 2 days and for 4 days if the samples are stored at 4˚C. "Median intra-day and inter-day CV [coefficient of variation] range from 2.9% to 7.8% (n=30) and 7.3% to 12.4% (n=66), respectively. The 2-tiered index score is reproducible over 4 consecutive days upon storage of blood at 4°C. A total of 2,888 three-level quality control data were collected from six flow cytometers with an overall failure rate below 3%. Median EC4d level is six net MFI (Interquartile [IQ] range 4-9 net MFI) and median BC4d is 18 net MFI (IQ range 13-27 net MFI) among 86,852 specimens submitted for testing. The incidence of 2-tiered positive test results is 13.4%" (Dervieux et al., 2017).

Putterman et al. (2014) compared the performance of C4d CB-CAPs on erythrocyte and B cells with antibodies to dsDNA, C3, and C4 in patients with SLE. A total of 794 individuals participated in this study, which included 205 healthy controls, 304 patients with SLE, and 285 patients with other rheumatic diseases. Both erythrocytes and B cells were measured with flow cytometry, and antibodies, including anti-dsDNA, were measured with solid-phase immunoassays. SLE activity was determined using the SLE Disease Activity Index Safety of Estrogens in Lupus National Assessment (SELENA) Modification, and the two-tiered AVISE Lupus test was developed. Results showed that "The combination of EC4d and BC4d in multivariate testing methodology with anti-dsDNA and autoantibodies to cellular and citrullinated antigens yielded 80% sensitivity for SLE and specificity ranging from 70% (Sjogren's syndrome) to 92% (rheumatoid arthritis) (98%vs. normal)" (Putterman et al., 2014).

Overall, the measurement of CB-CAPs was more sensitive for SLE diagnostic purposes than complement or anti-dsDNA measurements.

Ramsey-Goldman et al. (2020) evaluated the use of CB-CAPs, using flow cytometry, or a multianalyte assay panel (MAP) that includes CB-CAPs (e.g., AVISE Lupus) on patients with suspected SLE (n = 92) who fulfilled three classification criteria of the American College of Rheumatology (ACR). They also compared the data with individuals with established SLE (n = 53). At the initial visit, the individuals with suspected SLE had statistically higher positive CB-CAP (28%) or MAP results (40%) than individuals with established SLE. "In probable SLE, MAP scores of >0.8 at enrollment predicted fulfillment of a fourth ACR criterion within 18 months (hazard ratio 3.11, P<0.01)." The authors, who did acknowledge compensation from Exagen, conclude that "[a] MAP score above 0.8 predicts transition to classifiable SLE according to ACR criteria" (Ramsey-Goldman et al., 2020).

Clinical Utility and Validity

ANA, ENA, and SDLT

Oglesby et al. (2014) performed a cost-savings impact analysis on when the diagnosis of SLE is made and how it affects the clinical and economic outcomes. Using a claims database of claims made between January 2000 and June 2010, the authors separated individuals into two groups (n = 4166 per group) —early diagnosis (within six months of onset of symptoms) and late diagnosis (6 or more months after the onset of symptoms)—based upon an algorithm using a patient's ICD-9 diagnosis code(s) on the claim(s) and when SLE medications were dispensed. Additional propensity scores were matched using data based on "age, gender, diagnosis year, region, health plan type, and comorbidities". Results show that the early diagnosis group had lower rates of mild, moderate, and severe flares as well as lower rates of hospitalization as compared to the late diagnosis group. Moreover, "[c]ompared with the late diagnosis patients, mean all-cause inpatient costs PPPM [per patient per month] were lower for the early diagnosis patients (US$406 vs. US$486; p = 0.016). Corresponding SLE-related hospitalization costs were also lower for early compared with late diagnosis patients (US$71 vs US$95; p = 0.013)." The values are adjusted to 2010 US dollars. The authors note that the other resource use and cost categories were consistent, concluding "[p]atients diagnosed with SLE sooner may experience lower flare rates, less healthcare utilization, and lower costs from a commercially insured population perspective" (Oglesby et al., 2014).

A study by Yeo et al. (2020) demonstrates that there is little benefit to repeat ANA testing if the initial test was negative by evaluating the cost of repeat ANA testing. From 2011 to 2018, 36,715 ANA tests were performed for 28,840 patients at a total cost of $675,029. Of these tests, 21.4% were repeats in which 54.9% of the patients initially tested negative. Of those who tested negative and repeated ANA testing, only 19% of the patients had a positive result when the test was repeated once in under two years, and this positive test did not lead to a change in diagnosis. Therefore, the authors conclude that "repeat ANA testing after a negative result has low utility and results in high cost" (Yeo et al., 2020).

Deng et al. (2016) investigated the clinical utility of ANA testing through different assays to see which one was most appropriate for evaluating patients with CTD. With 1000 samples collected, they compared an enzyme immunoassay (EIA), immunofluorescence assay (IFA), and multiplex immunoassay (MIA) in terms of specificity and sensitivity of testing. The researchers found that through using weights to define a patient sample that reflected the intended testing population and a normalized specificity of 90% to standardize the comparison between tests, the MIA, EIA, and IFA had sensitivities of 67%, 67%, and 56%, respectively. However, with a varying clinical cutoff, the IFA could obtain a sensitivity of 94% and a corresponding specificity of only 43%. This demonstrated that the sensitivity and specificity could easily vary with predetermined cutoffs; but, there were "no statistically significant differences in the clinical utility of the IFA, EIA, or MIA" (Deng et al., 2016).

Alsaed et al. (2021) compared the performance of ANA testing via ELISA vs IIF for CTDs. From a sample of 1457 patients and 12,439 tests ordered in 2016, they found that with "cut-off ratio ≥ 1.0 for ANA-ELISA and a dilutional titre ≥ 1:80 for ANA-IIF, the sensitivity of ANA-IIF and ANA-ELISA for all CTDs were 63.3% vs 74.8% respectively. For the SLE it was 64.3% vs 76.9%, Sjogren's Syndrome was 50% vs 76.9% respectively. The overall specificity of ANA-ELISA was 89.05%, which was slightly better than ANA-IIF 86.72%". This communicated the ELISA was slightly better than IIF in sensitivity and specificity, which could influence the convention of using IIF going forward if these findings are reflected in other cohort studies.

Biomarker analysis

Wallace et al. (2019) performed a randomized prospective trial to assess the clinical utility of the AVISE lupus MAP test (MAP/CB-CAP) as compared to standard diagnosis laboratory testing (SDLT). A total of 145 patients with a history of positive antinuclear antibody status were randomly assigned to either an SDLT arm (n = 73) or the MAP/CB-CAP arm (n = 72) of the study. Treatment changes were recorded based on either the SDLT or MAP/CB-CAP results. Even though the demographics between the two arms of the study were similar, the results were different. "Post-test likelihood of SLE resulting from randomisation in the MAP/CB-CAPs testing arm was significantly lower than that resulting from randomisation to SDLT arm on review of test results (−0.44±0.10 points vs −0.19±0.07 points) and at the 12-week follow-up visit (−0.61±0.10 points vs −0.31±0.10 points) ($p<0.05$). Among patients randomised to the MAP/CB-CAPs testing arm, two-tiered positive test results associated significantly with initiation of prednisone ($p=0.034$)" (Wallace et al., 2019). The authors conclude that testing such as the AVISE Lupus test has clinical utility and does affect treatment decisions.

A longitudinal, retrospective study by Mossell et al. (2016) of 46 patients who were anti-nuclear antibodies (ANA) positive but SLE-specific autoantibodies negative was conducted to evaluate the clinical utility of the AVISE Lupus test. 23 of the patients were in the "case" group (i.e. positive result based on the AVISE Lupus test), and 23 patients were in the "control" or negative results group. The charts of each individual were reviewed at two different times: T0 (or the initial time) and T1 (or approximately one year later). The case group was diagnosed with SLE at a higher rate than the control group (87% vs. 17%, respectively); moreover, the case group fulfilled four of the ACR classification criteria of SLE at a higher rate than the control

group (43% vs 17%, respectively). The authors found that the sensitivity of the AVISE Lupus test (83%) is statistically significantly higher than the ACR score (42%, p = 0.006). Even at the initial baseline, individuals in the case group were prescribed anti-rheumatic medications more frequently (83% vs. 35%, p = 0.002) than the control group, indicating that a positive AVISE Lupus test may result in a more aggressive early treatment therapy (Mossell et al., 2016).

Liang et al. (2020) assayed the utility of the AVISE test in predicting lupus diagnosis and progression in 117 patients who previously did not have a diagnosis of SLE. The study assessed the patients at the time of the initial AVISE test (t = 0) and two years later (t = 2) using the SLE diagnosis criteria of the Systemic Lupus International Collaborating Clinics (SLICC) and ACR and the SLICC Damage Index (SDI) to measure SLE damage. After two years, patients who tested positive developed SLE at a significantly higher rate than those who tested negative using the AVISE test (65% vs 10.3%, p < 0.0001). AVISE-positive patients have more SLE damage after two years than AVISE-negative patients (1.9±1.3 vs 1.03±1.3, p=0.01). In particular, the authors note that the levels of BC4d "correlated with the number of SLICC criteria at t=0 (r=0.33, p< 0.0001) and t=2 (r=0.34, p<0.0001), as well as SDI at t=0 (r=0.25, p=0.003) and t=2 (r=0.26, p=0.002)" (Liang et al., 2020).

Alexander et al. (2021) further validated the clinical utility of the AVISE lupus test via a systematic review of medical records of ANA-positive patients with positive (>0.1) or negative (<-0.1) MAP scores. They found that the "odds of higher confidence in SLE diagnosis increased by 1.74-fold for every unit increase of the MAP score" with statistical significance, demonstrating that the test still further solidifies a diagnosis of SLE and can help inform "appropriate treatment decisions" (Alexander et al., 2021).

A study by Clarke et al. (2020) demonstrates the cost-effective management of systemic lupus erythematosus (SLE) using a MAP rather than SDLTs. The higher specificity of MAP allows for an earlier SLE diagnosis, prompt initiation of the appropriate therapy, and fewer unnecessary and costly hospitalizations or investigations. Current SDLTS, such as ANA tests, have a high diagnostic sensitivity, but a high false-positive rate. MAP combines complement C4d activation products on erythrocytes and B cells with SDLTs, with antibodies to nuclear antigens, dsDNA IgG (with Crithidia confirmation), Smith, Sjogren's syndrome type-B (SS-B/La), topoisomerase I (Scl-70), centromere protein B (CENP), histidyl t-RNA synthetase (Jo-1), and cyclic citrullinated peptites (CCP) to improve SLE diagnosis. MAP "yields improved overall diagnostic performance with a sensitivity and specificity of 80% and 86%, respectively, compared with a sensitivity and specificity of 83% and 76%, respectively, for SDLTs. Despite the lower sensitivity, the superior specificity of MAP (86%) over SDLTs (76%) results in a higher positive predictive value associated with MAP (36.75%) compared with SDLTs (26.02%)" (Clarke et al., 2020). The improved specificity of MAP resulted in a cost savings of $1,991,152 to a US commercial plan over a 4-year time horizon, which translates to $0.04 in per member per month (PMPM) savings (Clarke et al., 2020).

Clinical validation of PrismRA was conducted in the Comparative Effectiveness Registry to Study Therapies for Arthritis and Inflammatory Conditions (CERTAIN) study (Bergman et al., 2020; Mellors et al., 2020). The CERTAIN trial was conducted by the Consortium of

Rheumatology Researchers of North America which consisted of 43 sites and 117 rheumatologists (Mellors et al., 2020). This prospective study analyzed baseline RNA sequencing and clinical assessments to determine the effectiveness of PrismRA to predict TNFi non-response. Evaluation of the clinical response to TNFi was performed at six months and was determined by ACR50. The CERTAIN study built and validated the biomarker panel used for MSRC analyses. The study found that PrismRA demonstrated a positive predictive value of 89.7%, a specificity of 86.8%, and a sensitivity of 50% (Mellors et al., 2020; Pappas et al., 2021).

Inadequate TNFi response predictions were further validated on integrated blood samples from CERTAIN and NETWORK-004 studies. NETWORK-004 was a 24-week blinded prospective study conducted at 73 sites to evaluate the ability of MSRC to identify TNFi non-responders at three and six months by ACR50 (evaluations were also conducted using other scales such as Disease Activity Score (DAS28)-CRP, and Clinical Disease Activity Index). CERTAIN samples were used for transcript biomarker feature selection (n=100) and cross validation of MSRC (n=245). In the NETWORK-004 cohort, MSRC validation was performed in samples from naïve (n=146) and TNFi exposed (n=113) patients. ACR50 of patients stratified by MSRC at six months according to prediction of an inadequate response to TNFi therapy had an odds ratio of 4.1 (95% CI 2.0–8.3; p value=0.0001). Patients with a non-response MSCR were 26 times less likely to achieve remission evaluated three months after TNFi therapy (Cohen et al., 2021). Both studies found that PrismRA was able to accurately predict TNFi non-responders according to multiple clinically validated measurement scales (Cohen et al., 2021; Mellors et al., 2020).

Bergman et al. (2020) performed modeling of the projected improvements from PrismRA and determined that ACR50 improved in the stratified cohort (40%) compared to the unstratified patient cohort (30%) and decreased costs of ineffective treatment by 19%. Further, PrismRA was shown to be a better predictor of inadequate response to TNFi treatment than clinical metrics alone (Bergman et al., 2020). Pappas et al. (2021) conducted a 32-question decision-impact survey involving 248 rheumatologists to determine whether predictive tests such as PrismRA appear to have clinical utility in RA patients' ability to respond to TNFi therapy. The study demonstrated that rheumatologists overwhelmingly supported the clinical need of predictive technologies to determine whether RA patients would respond to TNFi therapies and that payers should provide coverage of predictive technology (Pappas et al., 2021).

According to Curtis et al. (2012), the MBDA algorithm (Vectra DA) was developed by screening 396 candidate biomarkers. An algorithm was then created to generate a composite score based on the 12 biomarkers most correlated to RA clinical disease activity which are as follows:

- Interleukin-6 [IL-6]
- Tumor necrosis factor receptor type I [TNFRI]
- Vascular cell adhesion molecule 1 [VCAM-1]
- Epidermal growth factor [EGF]
- Vascular endothelial growth factor A [VEGF-A]
- YKL-40
- Matrix metalloproteinase 1 [MMP-1]

- MMP-3
- CRP
- Serum amyloid A [SAA]
- Leptin
- Resistin

These biomarkers represent several processes related to RA, such as cartilage remodeling and cytokine signaling pathways. A score of ≤29 is considered "low" activity, between 29 and 44 is "moderate" activity, and >44 is "high" activity. The MBDA is intended to provide separate information from a clinical evaluation of joints and should be used as a complement, not as a replacement (Curtis et al., 2012).

This MBDA has been shown to correlate significantly ($r=0.72$; $p<0.001$) with a disease activity score based on the 28-joint Disease Activity Score based on CRP (DAS28-CRP) and has been validated for clinical use as a disease activity marker in RA (Curtis et al., 2012). Both Hirata et al. (2013) and Bakker et al. (2012) found the MBDA score to correlate well with disease activity and could complement other existing measures of RA assessment. Remission based on the MBDA score was a significant predictor of radiographic non-progression, whereas both remission-defined DAS28-CRP and American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) criteria was not. The MBDA test was also useful in assessing the risk of radiographic progression among patients who met clinical remission criteria. MBDA results may provide an important addition to clinical assessment, however, further studies are needed to confirm its clinical utility in the management of RA (van der Helm-van Mil et al., 2013).

Li et al. (2013) evaluated the impact of an MBDA blood test for rheumatoid arthritis (RA) on treatment decisions made by six health care providers (HCPs) in 101 patients. HCPs completed surveys before and after viewing the MBDA test result, recording dosage and frequency for all RA medications and assessment of disease activity. Frequency and changes in treatment plan that resulted from viewing the MBDA test result were determined. The MBDA test results were found to have changed 38% of patients' treatment plans. Furthermore, treatment plans were changed 63% of the time the MBDA test results were found to be "not consistent" or "somewhat consistent" with the clinical assessment of disease activity. However, any improvement in clinical outcomes caused was not reported, and the overall amount of drug use was not affected (Li et al., 2013).

Another study by Li et al. (2016) assessed the correlation between MBDA score and disease progression in 163 RA patients. The study found that low radiographic progression was associated with low MBDA scores, and higher scores were associated with more frequent and severe progression. Notably, MBDA scores correlated with progression even when a conventional measure such as the DAS28 indicated otherwise. For example, low risk of progression was associated with a low MBDA score, even when a concurrent DAS28 score was high. The authors concluded that MBDA may be a good complement for conventional measures, as well as provide information on changing treatment plans (Li et al., 2016).

Curtis et al. (2018) initially studied the influence of age, obesity, and other comorbidities on the MBDA test. A cross-sectional analysis of RA patients who have participated in an MBDA test was used (n=357). "Of 357 eligible patients, 76% (n = 273) had normal CRP (<10mg/L) with high (33%), moderate (45%), and low (22%) disease activity by MBDA. The MBDA score was significantly associated with BMI, age, CDAI [clinical disease activity index], and SJC [swollen joint count]" (Curtis et al., 2018). Almost one third of participants had normal CRP scores but high MBDA scores. "In this real-world analysis, the MBDA score was associated with RA disease activity, obesity, and age, and was negligibly affected by common comorbidities" (Curtis et al., 2018). The authors conclude by suggesting that an adjusted MBDA score may require development to account for BMI and age. Such a study was then published the following year. Curtis et al. (2019) developed an MBDA test that will include additional factors such as sex, age and obesity in RA patients. Obesity, or adiposity, was measured using either BMI or serum leptin concentration. Two cohorts were studied, totaling 1736 patients. Overall, the authors have developed "a leptin-adjusted MBDA score that has significantly improved [the] ability to predict clinical disease activity and radiographic progression" (Curtis et al., 2019). It was suggested that this leptin-adjusted MBDA score "significantly adds information to DAS28-CRP and the original MBDA score in predicting radiographic progression. It may offer improved clinical utility for personalized management of RA" (Curtis et al., 2019).

A recent study analyzed the measurement of serum biomarkers at early RA disease onset in hopes to better predict disease progression (Brahe et al., 2019). MBDA score and changes in this score were evaluated to predict DAS28-CRP remission. A total of 180 patients participated in this study and were treated with either methotrexate and adalimumab (n = 89) or methotrexate and placebo (n = 91) in addition to a glucocorticoid injection into swollen joints; results showed that "Early changes in MBDA score were associated with clinical remission based on DAS28-CRP at 6 months" (Brahe et al., 2019).

In a study by Ma et al. (2020), the MBDA test was used to explore the role of biomarkers in predicting remission of RA. Serum samples for 148 patients were assessed for MBDA score at three months, six months, and at one year. RA patients on greater than six months stable therapy in stable low disease activity were assessed every three months for one year. Patients not fulfilling any remission criteria at baseline were classified as 'low disease activity state' (LDAS). Patients not fulfilling any remission criteria over one year were classified as 'persistent disease activity' (PDA). Of the 148 patients, 27% were in the LDAS group and over one year and 9% of patients were classified as PDA. Baseline MBDA score and concentrations of IL-6, leptin, SAA and CRP were significantly lower in all baseline remission criteria groups in comparison to LDAS groups. The individual MBDA biomarkers (IL-6, leptin, SAA, CRP) and initial MBDA score was able to differentiate between remission at baseline and LDAS. The authors state that these findings highlight the potential value of repeated measurements of MBDA score to evaluate the stability of clinical disease activity over time (Ma et al., 2020).

In a combined analysis of the OPERA, SWEFOT, and BRASS studies in which a newer version of the MBDA score was validated, Curtis analyzed the prognostic value of the adjusted MBDA score for radiographic progression in RA. The new MBDA score, used in these three studies, adjusts for age, sex, and adiposity. Curtis evaluated associations of radiographic progression

(ΔTSS) per year with the adjusted MBDA score, seropositivity, and clinical measures using linear and logistic regression. The adjusted MBDA score was validated in SWEFOT, compared with the other two cohorts, and used to generate curves for predicting risk of radiographic progression. The adjusted MBDA score was found to be the "strongest, independent predicator of radiographic progression (ΔTSS > 5) compared with seropositivity (rheumatoid factor and/or anti-CCP), baseline TSS, DAS28-CRP, CRP SJC, or CDAI. Its prognostic ability is not significantly improved by the addition of DAS28-CRP, CRP, SJC, or CDAI" (Curtis et al., 2021).

Fleischmann et al. (2022) engaged in a multicenter, randomized, placebo-controlled trial of repository corticotropin injection (RCI) in patients with active RA. The utility of an MBDA score was measured against the utility of the Disease Activity Score to assess disease activity in RA. Study participants received 80 units of RCI twice weekly, and those who had low disease activity at week 12 were given either 80 units of RIC or a placebo twice weekly. The changes in disease activity (measured by DAS28-ESR, CDAI, and MBDA scores) were analyzed, including correlations between MBDA scores and both DAS28-ESR and CDAI scores. Results showed "changes from baseline in DASw8-ESR and CDAI scores suggested the RCI therapy led to clinically meaningful improvements in disease activity, but improvements from baseline in MBDA scores were below the minimally important difference threshold." The authors concluded that MBDA scores were not "sufficiently responsive" in the assessment of RA disease activity. The authors also said that MBDA should not be used as a preferred disease activity measure for RA patients (Fleischmann et al., 2022).

Guidelines and Recommendations

American College of Rheumatology

Systemic Lupus Erythematosus (SLE)

In 1997, the Diagnostic and Therapeutic Criteria Committee of the ACR revised the 1982 criteria for SLE. Often referred to as the 1997 ACR criteria, these revisions included the addition of "[p]ositive finding of antiphospholipid antibodies based on 1) an abnormal serum level of IgG or IgM anticardiolipin antibodies, 2) a positive test result for lupus anticoagulant using a standard method, or 3) a false-positive serologic test for syphilis known to be positive for at least six months and confirmed by *Treponema pallidum* immobilization or fluorescent treponemal antibody absorption test" (Hochberg, 1997). The 1997 ACR criteria consists of 11 possible different criterion and each criterion may have more than one definition. A minimum score of four out of 11 is indicative of SLE. According to the Centers for Disease Control and Prevention (CDC), rheumatologists can use these criteria "to classify SLE for research purposes"(CDC, 2022). The 1997 ACR criteria in a study by Mosca et al. (2019), using a cohort of 616 patients, has a reported accuracy of 75.5%, sensitivity of 66.1%, and specificity of 91.6%. The criteria are as follows (ACR, 1997; CDC, 2022):

1. Malar Rash
2. Discoid Rash

3. Photosensitivity
4. Oral Ulcers
5. Nonerosive Arthritis
6. Pleuritis or Pericarditis
7. Renal Disorder
8. Neurologic Disorder
9. Hematologic Disorder
10. Immunologic Disorder
11. Positive Antinuclear Antibody

The ACR published a statement on the Methodology of Testing for Antinuclear Antibodies (ACR, 2015) which states:

1. The ACR supports the immunofluorescence antinuclear antibody (ANA) test using Human Epithelial type 2 (HEp-2) substrate, as the gold standard for ANA testing.
2. Hospital and commercial laboratories using alternative bead-based multiplex platforms or other solid phase assays for detecting ANAs must provide data to ordering healthcare providers on request that the alternative assay has the same or improved sensitivity compared to IF ANA.
3. In-house assays for detecting ANA as well as anti-DNA, anti-Sm (anti-Smith antibodies), anti-RNP (antinuclear ribonucleoprotein), anti-Ro/SS-A (anti-Ro/Sjogren Syndrome-A), anti La/SS-B (anti-La/Sjogren Syndrome-B), etc., should be standardized according to national (e.g., CDC) and/or international (e.g., WHO, IUIS) standards.
4. Laboratories should specify the methods utilized for detecting ANAs when reporting their results.

The above positions were reaffirmed in 2019 (ACR, 2024).

The ACR, together with "Choosing Wisely" also developed a list of five tests, treatments or services that are commonly used in rheumatology practice, but their value should be questioned. The ANA testing was the first on the final top five items list with level of evidence Grade 1C. In their review, the Task Force considered recommendations currently published by American College of Pathologists (ACP), ACR, and Italian Society of Laboratory Medicine (ISLM). They have issued the following recommendation: "Do not test antinuclear antibody (ANA) subserologies without a positive ANA and clinical suspicion of immune-mediated disease" (Yazdany et al., 2013). For their list of five things to question for pediatric rheumatology, two points pertain to ANA testing (Rouster-Stevens et al., 2014). "Do not order autoantibody panels unless positive ANAs and evidence of rheumatic disease. There is no evidence that autoantibody testing (including ANA and autoantibody panels) enhances the diagnosis of children with musculoskeletal pain in the absence of evidence of rheumatic disease as determined by a careful history and physical examination." The latter recommendation also stated, "Do not repeat a confirmed positive ANA in patients with established JIA [juvenile idiopathic arthritis] or SLE" (Rouster-Stevens et al., 2014).

These guidelines were reviewed and reaffirmed in 2021.

Rheumatoid Arthritis

In 2021, the ACR released an updated guideline on the management of rheumatoid arthritis, including new recommendations for high-risk groups. Pertaining to disease management and the risk of hepatotoxicity associated with methotrexate therapy, the ACR notes that "the use of methotrexate should be restricted to patients with normal liver enzymes and liver function tests without evidence of liver disease or liver fibrosis." No multi-biomarker tests or disease activity tests (such as Vectra DA or PrismRA) were mentioned in the guideline for diagnostic or disease management indications (Fraenkel et al., 2021).

European League Against Rheumatism/American College of Rheumatology (EULAR/ACR)

Systemic Lupus Erythematosus (SLE)

The EULAR/ACR published a joint guideline to develop new classification criteria for systemic lupus erythematosus (SLE). In it, they stated that antinuclear antibodies (ANA) "at a titer of ≥1:80 on HEp-2 cells or an equivalent positive test" was to be an "entry criterion": if absent, the condition is not SLE; if present, apply additive criteria such as leukopenia or oral ulcers. Antiphospholipid antibodies, complement proteins, and SLE-specific antibodies (anti-dsDNA antibodies, Anti-Smith antibodies) are all included as additive criteria for SLE diagnosis (Aringer et al., 2019).

Rheumatoid Arthritis

In 2022, an international task force was formed to address the safety and efficacy of disease-modifying antirheumatic drugs (DMARDs) and glucocorticoids (GCs) in the treatment of Rheumatoid Arthritis. The guideline focuses on treatment concerns. Regarding "biomarkers" they caution that certain biomarkers – i.e., acute phase reactants (APRs) such as CRP and other biomarkers comprising APRs "may respond independently of clinical improvement when antibodies to the IL-6 receptors, JAK inhibitors and even TNF-inhibitors are used." The guideline does not mention multi-biomarker and disease activity tests such as Vectra DA or PrismRA (Josef et al., 2023).

Systemic Lupus International Collaborating Clinics (SLICC)

The 2012 SLICC Classification Criteria for SLE splits the 17 criteria into two divisions—either clinical or immunologic. An individual scoring at least a 4, including at least one clinical criterion and one immunologic criterion, is classified as having SLE. The criteria are cumulative and do not need to be concurrently expressed or present (Petri et al., 2012). Mosca et al. (2019) also analyzed the accuracy and validity of the SLICC classification criteria, using a cohort of 616 patients, reporting an accuracy of 83.1%, sensitivity of 83.5%, and specificity of 82.4%. The criteria include the following (Petri et al., 2012):

A. Clinical Criteria

1. Acute cutaneous lupus, such as lupus malar rash or subacute cutaneous lupus
2. Chronic cutaneous lupus, such as classic discoid rash or discoid lupus/lichen planus overlap
3. Nonscarring alopecia
4. Oral or nasal ulcers
5. Joint disease
6. Serositis
7. Renal criteria, such as urine protein-to-creatinine ratio representing 500 mg protein/24 hours or red blood cell casts
8. Neurologic criteria, such seizures, psychosis, myelitis, and so on
9. Hemolytic anemia
10. Leukopenia or lymphopenia
11. Thrombocytopenia

B. Immunologic Criteria

1. ANA
2. Anti-dsDNA
3. Anti-Sm
4. Antiphospholipid antibodies
5. Low complement (Low C3, Low C4, or Low CH50)
6. Direct Coombs test in the absence of hemolytic anemia

British Columbia Rheumatoid Arthritis

The BC Rheumatoid Arthritis guideline includes a table of factors used in the diagnosis of rheumatoid arthritis. The C-Reactive Protein (CRP) or Erythrocyte Sedimentation Rate (ESR) test is noted as the "preferred test," CRP/ESR indicates only inflammatory process but the guideline notes "low specificity." For RF, "RF has low sensitivity and specificity for RA. Seropositive RA has a worse prognosis than seronegative RA." Regarding anti-CCP, they write, "Anti-cyclic citrullinated protein antibodies (Anti-CCP) may have some value."

For disease activity monitoring, "CRP is more sensitive to short term fluctuations" and "ESR elevated in many but not all with active inflammations." Concerning monitoring, Rheumatoid Factor Latex Test (RF), "RF has low sensitivity and specificity for RA. Seropositive RA has a worse prognosis than seronegative RA" (British Columbia Rheumatoid Arthritis, 2012).

National Institute for Clinical Excellence (NICE)

In a section on referral, diagnosis and investigations, NICE recommends:

- "Refer for specialist opinion anyone with suspected persistent synovitis of undetermined cause. Refer urgently even if blood tests show a normal acute-phase response or negative rheumatoid factor and if:
  - The small joints of the hands or feet are affected
  - More than one joint is affected, or

- There has been a delay of three months or longer between symptom onset and seeking medical advice.

[Based on high and moderate quality observational studies of early prognosis and identification or diagnosis]."

- "Offer to test for rheumatoid factor in people with suspected rheumatoid arthritis who have synovitis. [Based on high and moderate quality early identification observational studies]
  - Consider measuring anticyclic citrullinated peptide antibodies in people with suspected rheumatoid arthritis if:
    - They are negative for rheumatoid factor, and
    - Combination therapy is being considered (see section on disease modifying antirheumatic drugs)" (Deighton et al., 2009).

The Royal Australian College of General Practitioners (RACGP)

The RACGP provides a recommendation on diagnosing those with suspected rheumatoid arthritis: "RECOMMENDATION 4 – DIAGNOSTIC INVESTIGATIONS (Grade A)
For patients presenting with painful and swollen joints, GPs should support clinical examination with appropriate tests to exclude other forms of arthritis and other differential diagnoses, and to predict patients likely to progress to erosive disease. Base investigations should include:

- erythrocyte sedimentation rate (ESR) and/or C-reactive protein (CRP)
- rheumatoid factor (RhF) and anti-cyclic citrullinated peptide (anti-CCP) antibody levels" (RACGP, 2009).

Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

Applicable CPT/HCPCS Procedure Codes

CPT	CPT Description

81490	Autoimmune (rheumatoid arthritis), analysis of 12 biomarkers using immunoassays, utilizing serum, prognostic algorithm reported as a disease activity score Proprietary test: Vectra® DA Lab/Manufacturer: Crescendo Bioscience, Inc.
81599	Unlisted multianalyte assay with algorithmic analysis
86038	Antinuclear antibodies (ANA)
86039	Antinuclear antibodies (ANA); titer
86200	Cyclic citrullinated peptide (CCP), antibody
86225	Deoxyribonucleic acid (DNA) antibody; native or double stranded
86235	Extractable nuclear antigen, antibody to, any method (eg, nRNP, SS-A, SS-B, Sm, RNP, Sc170, J01), each antibody
86430	Rheumatoid factor; qualitative
86431	Rheumatoid factor; quantitative
0039U	Deoxyribonucleic acid (DNA) antibody, double stranded, high avidity Proprietary test: Anti-dsDNA, High Salt/Avidity Lab/Manufacturer: University of Washington, Department of Laboratory Medicine/Bio-Rad
0062U	Autoimmune (systemic lupus erythematosus), IgG and IgM analysis of 80 biomarkers, utilizing serum, algorithm reported with a risk score Proprietary test: SLE-key® Rule Out Lab/Manufacturer: Veracis Inc
0312U	Autoimmune diseases (eg, systemic lupus erythematosus [SLE]), analysis of 8 IgG autoantibodies and 2 cell-bound complement activation products using enzyme-linked immunosorbent immunoassay (ELISA), flow cytometry and indirect immunofluorescence, serum, or plasma and whole blood, individual components reported along with an algorithmic SLE-likelihood assessment Proprietary test: Avise® Lupus Lab/Manufacturer: Exagen Inc

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

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Revision History

Revision Date	Summary of Changes
03/06/2024	Reviewed and Updated: Updated the background, guidelines and recommendations, and evidence-based scientific references. Literature review necessitated the following changes in coverage criteria: CC1, CC6 edited for clarity and consistency. Addition of new CC3: "3) For individuals with painful and swollen joints and a clinical suspicion of rheumatoid arthritis, testing for rheumatoid factor (RF) and/or anti-cyclic citrullinated peptide (anti-CCP) antibodies MEETS COVERAGE CRITERIA: a) Once per lifetime in individuals with stable symptoms. b) Repeat testing only if a significant change in symptoms occurs." Added new CPT code 86200, 86430, 86431
03/01/2023	Reviewed and Updated: Updated the background, guidelines and recommendations, and evidence-based scientific references. Literature review necessitated the following changes in coverage criteria: Titled changed to "Biomarker Testing for Autoimmune Rheumatic Disease" Addition of "a) Once per lifetime in individuals with stable symptoms. b) Repeat testing only if a significant change in symptoms occurs." To CC1, now reads: "1) For individuals with a high clinical suspicion of autoimmune disease, testing for antinuclear antibodies (ANA) MEETS COVERAGE CRITERIA. a) Once per lifetime in individuals with stable symptoms. b) Repeat testing only if a significant change in symptoms occurs. Information from G2127 wrapped into this policy, resulting in a new CC9: "9) For the management of rheumatoid arthritis (RA), serum biomarker panel testing (e.g., Vectra DA score, PrismRA) DOES NOT MEET COVERAGE CRITERIA." All CC other than CC5 edited for clarity and consistency. Added CPT 81490

03/09/2022	Annual review: Updated background, guidelines and recommendations, and evidence-based scientific references. Literature review did not necessitate modification to coverage criteria. CPT Changes: Added PLA code 0312U.
03/03/2021	Annual review: Updated definition, background, guidelines and recommendations and evidence-based scientific references. Literature review did not necessitate any modification to coverage criteria.
12/02/2020	Off cycle review: Updated description, background, guidelines and recommendations, and evidence-based scientific references. Literature review necessitated the following modifications:
03/10/2020	Annual Review: Updated description, background, guidelines and recommendations, and evidence-based scientific references. Reordered CCs according to AHS protocol. Switched E&I to DNMCC with the preceding statement regarding lack of scientific literature. Literature review necessitated the following modification to the coverage criteria: • Added CC stating that testing of ANA and/or ENA DNMCC in individuals during wellness visits or general encounters without abnormal findings.
03/01/2019	Annual Review: Updated description, background, guidelines, and evidence-based scientific references. Reordered the CCs according to AHS protocol (i.e. MCC listed first, DNMCC listed next, E&I listed last). Reworded CC concerning ENA: "ENA panel testing of specific autoantibodies such as nRNP, SS-A, SS-B, Sm, RNP, Sc170, or Jo1 **MEETS COVERAGE CRITERIA** in patients with raised antibody titer or abnormal immunological findings in serum and clinical correlation with the appropriate autoimmune disorder". Added CC that testing of specific antibodies when ANA is either negative or low positive **DNMCC** except for anti-Jo-1 in myositis and anti-SSA for lups or Sjorgren's syndrome. Added 81599, 0030U and 0063U
03/16/2018	Annual review: Definition, Background, Guidelines and Recommendations and Evidence-based Scientific References were updated. Literature review did not necessitate any modification to coverage criteria. No changes to coding.
02/12/2017	Off cycle review: Terminology revised in CC #2: Changed from "is investigational and does not meet coverage criteria" to "is not medically
12/12/2016	Annual review completed. Coverage criteria revised: • CC #1 - removed "history and physical" • CC #5 – deleted content re: diagnosis of anti-phospholipid syndrome New CC added to address investigational status for panel testing with proprietary algorithms and/or index scores
11/16/2015	Initial presentation

Biomarkers for Myocardial Infarction and Chronic Heart Failure

Policy Number: AHS – G2150 – Biomarkers for Myocardial Infarction and Chronic Heart Failure	Prior Policy Name and Number, as applicable: AHS-G2150- Cardiac Biomarkers for Myocardial Infarction
Initial Presentation Date: 4/19/2018 Revision Date: February 1, 2025	

Policy Description

Cardiac biomarkers are the biochemical markers released in blood from injured myocardial tissue in both acute and chronic conditions, such as myocardial infarction or heart failure. They become elevated in blood after a certain period and can be measured. Examples of cardiac biomarkers commonly used in the acute clinical setting include troponin and creatine kinase MB isoenzyme (CKMB) (Thygesen et al., 2007). Others, such as suppression of tumorigenicity 2 (ST2), can serve in long-term as markers of cardiomyocyte stress and fibrosis for risk stratification of patients with a wide spectrum of cardiovascular diseases (Bayes-Genis et al., 2015).

Related Policies

Policy Number	Policy Title
AHS-G2050	Cardiovascular Disease Risk Assessment

Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) For individuals presenting with signs and symptoms of acute coronary syndrome (see Note 1), **quantitative** measurement of cardiac troponin (troponin T or I) for the diagnosis of myocardial infarction (MI) (when tested at an outpatient facility capable of performing an adequate clinical MI evaluation) **MEETS COVERAGE CRITERIA** up to four times within the first 72 hours following initial presentation.

2) For individuals presenting with signs and symptoms of acute coronary syndrome (see Note 1), measurement of following cardiac biomarkers for the diagnosis and/or prognosis of MI **DOES NOT MEET COVERAGE CRITERIA**:

   a) Aspartate aminotransferase (AST/SGOT).

   b) Cardiac creatine kinase isoenzyme MB (CKMB).

   c) Creatine kinase (CK).

   d) Creatine kinase isoenzymes.

   e) Lactate dehydrogenase (LD, LDH).

   f) Myoglobin.

3) Measurement of B-type natriuretic peptide (BNP) **or** N-terminal proBNP (NT-proBNP) **MEETS COVERAGE CRITERIA** in **any** of the following situations:

   a) To diagnose heart failure in individuals presenting with dyspnea.

   b) To establish disease severity in individuals with chronic heart failure (up to four times per year in the outpatient setting).

4) For individuals presenting with signs and symptoms of acute coronary syndrome (see Note 1), measurement of cardiac biomarkers in an outpatient setting which is not capable of performing adequate clinical MI evaluation (e.g., independent lab or physician's office) **DOES NOT MEET COVERAGE CRITERIA**.

5) In the outpatient setting, **qualitative** measurement of cardiac troponin (troponin T or I) **DOES NOT MEET COVERAGE CRITERIA.**

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

6) For individuals presenting with signs and symptoms of acute coronary syndrome (see Note 1), measurement of the following cardiac biomarkers for the diagnosis and/or prognosis of MI **DOES NOT MEET COVERAGE CRITERIA**:

   a) Copeptin.

   b) Troponin C.

   c) C-reactive protein.

   d) Heart-type fatty acid binding protein (H-FABP).

   e) Any other cardiac biomarkers not listed above.

7) For all situations in the outpatient setting, analysis of ST2 and/or its isoforms (e.g., Presage ST2) **DOES NOT MEET COVERAGE CRITERIA.**

NOTES:

Note 1: *Acute Coronary Syndrome/Myocardial Infarction Common Signs and Symptoms (Reeder, 2023):*

- Ischemic chest pain with radiation to an upper extremity, radiation to both arms, and pain associated with diaphoresis or with nausea and vomiting.
- Squeezing, tightness, pressure, constriction, crushing, strangling, burning, heartburn, fullness in the chest, band-like sensation, knot in the center of the chest, lump in throat, ache, heavy weight on chest and toothache (when there is radiation to the lower jaw).
- Ischemic pain often radiates to other parts of the body including the upper abdomen (epigastrium), shoulders, arms (upper and forearm), wrist, fingers, neck and throat, lower jaw and teeth (but not upper jaw), and not infrequently to the back (specifically the interscapular region).
- Shortness of breath, belching, nausea, indigestion, vomiting, diaphoresis, dizziness, lightheadedness, clamminess, and fatigue.

Atypical Signs and Symptoms (Reeder, 2023):

Dyspnea alone, weakness, nausea and/or vomiting, epigastric pain or discomfort, palpitations, syncope, or cardiac arrest.

Table of Terminology

Term	Definition
AATS	American Association for Thoracic Surgery
ACC	American College of Cardiology
ACS	Acute coronary syndrome Syndromes
ADH	Antidiuretic Hormone
AHA	American Heart Association
AMI	Acute myocardial infarction
APACE	Advantageous predictors of acute coronary syndrome evaluation
ASCP	American Society for Clinical Pathology
ASE	American Society of Echocardiography
ASNC	American Society of Nuclear Cardiology
AST	Aspartate aminotransferase
AUC	Appropriate use criteria
AUC	Area under the curve
AVP	Arginine vasopressin
B	Brain
BB	Bundle branch
BMI	Body mass index
B-NR	Level B – nonrandomized
BNP	Brain natriuretic peptide
CABG	Coronary artery bypass grafting
CCS	Canadian Cardiovascular Society

CHOPIN	Copeptin Helps in the Early Detection of Patients with Acute Myocardial Infarction
CK	Creatine kinase
CKMB	Creatine Kinase MB isoenzyme
CKMB	Cardiac Creatine Kinase isoenzyme MB
CKMM	Creatine kinase – skeletal muscle
CLIA '88	Clinical Laboratory Improvement Amendments of 1988
CMP	Cardiomyopathy
CMS	Centers For Medicare and Medicaid
CPK	Creatine phosphokinase
cTnI	Cardiac troponin I
cTnT	Cardiac troponin T
ECG	Electrocardiogram
ED	Emergency Department
ESC	European Society of Cardiology
FDA	Food and Drug Administration
GDF-15	Growth differentiation factor 15
HCPCS	Healthcare common procedure coding system
H-FABP	Heart-type fatty acid binding protein
HF	Heart failure
HFSA	Heart Failure Society of America
hs-cTn	High-sensitivity cardiac troponin
IL-1R	Interleukin 1 receptor
IL-2	Interleukin 2
IL-33	Interleukin 33
ILCOR	International Liaison Committee on Resuscitation
LAMP	*Leicester acute myocardial infarction peptide* gene
LBBB	Left bundle branch block
LD or LDH	Lactate dehydrogenase
LDTs	Laboratory-developed tests
LOE	Level of evidence
M	Muscle
MACE	Major adverse cardiovascular events
MI	Myocardial infarction
MINOCA	Myocardial infarction in the absence of obstructive coronary artery
NICE	National Institute for Health and Care Excellence
NSTE-ACS	Non-ST-elevation acute coronary syndromes
NSTEMI	Non-ST elevation myocardial infarction
NT-proBNP	N-terminal pro-B-type natriuretic peptide
PA	Prior authorization
PCI	Percutaneous coronary intervention
POC	Point of care
ROC	Receiver operating characteristic
SCAI	Society for Cardiovascular Angiography and Interventions
SCCT	Society for Cardiovascular Computed Tomography
SGOT	Serum glutamic-oxaloacetic transaminase

sST2	Soluble suppression of tumorigenicity 2
ST2	Soluble interleukin 1 receptor-like 1
ST2L	Transmembrane isoform of S2
STEMI	ST-elevation myocardial infarction
STS	Society of Thoracic Surgeons
ST-T	ST-segment-T Wave
T	Troponin
TIMI	Thrombolysis in myocardial infarction
TnC	Troponin C
TnI	Troponin I
TnT	Troponin T
ULN	Upper limit of normal
URL	Upper reference limit
WHF	World Heart Federation
WHO	World Health Organization

Scientific Background

Acute coronary syndromes (ACS) represent continuous events starting with angina, reversible injury, and progressing to unstable angina; these syndromes are frequently associated with minor myocardial damage, and myocardial infarction (MI) that results in extensive tissue necrosis (Thygesen et al., 2007). Patients with ACS usually present with chest pain and associated signs and symptoms. These patients are subdivided into two major categories based on the 12-lead electrocardiogram (ECG). If an ST-segment elevation is observed on the ECG, it is indicative of acute ST-elevation myocardial infarction (STEMI) type of ACS. If the ECG shows ST-segment depression, T-wave changes, or no ECG abnormalities, it is indicative of non-ST elevation myocardial infarction (NSTEMI) and unstable angina.

ACS is complex. However, the most common cause is atherosclerotic coronary artery disease with rupture of atherosclerotic plaque (Amsterdam et al., 2014). The first documented definition of acute MI was established in 1979 by the World Health Organization (WHO). It included in the criteria for MI diagnosis the recommendation to use the rise or fall patterns of cardiac biomarkers, such as creatine kinase (CK), creatine kinase's MB isoenzyme (CK-MB), lactate dehydrogenase (LDH) or aspartate aminotransferase (AST) activities (WHO, 1979). Since then, other societies have proposed their own criteria for diagnosis. The third universal definition of MI includes typical clinical symptoms, suggestive ECG changes, or imaging evidence of new loss of viable myocardium or new regional wall abnormality with a rise and/or fall of cardiac biomarkers (Thygesen et al., 2012). Nonetheless, the universal criteria are being refined by cardiovascular societies and will likely change with scientific progress and better understanding of MI pathophysiology.

Myocardial infarction results in cardiac injury and extensive tissue necrosis. The cellular membranes become compromised and release structural proteins and other macromolecules into cardiac interstitial space. These released markers are called cardiac biomarkers. The levels of these cardiac biomarkers in blood will rise and fall with time after MI (Thygesen et al., 2007). The first cardiac biomarker, aspartate aminotransferase (AST), was used for MI diagnosis in 1954. AST is present in human tissues as two isoenzymes: cytoplasmic and mitochondrial. AST is a non-specific biomarker, and its activity could also be elevated in other conditions, such as hepatic congestion secondary to congestive heart failure. Since then, other cardiac biomarkers were used as an aid in diagnosis of MI, but due to their non-specificity and other reasons, many of them are no longer used in clinical practice or their use remains very limited (Danese & Montagnana, 2016). The most common cardiac biomarkers and their characteristics are summarized in the table from (Danese & Montagnana, 2016):

Table 1 Characteristics of AMI biomarkers

Biomarker	First assay development, year	Molecular weight, Da	Kinetics			Sensitivity for myocyte necrosis	Specificity for myocyte necrosis
			First detection, hours	Maximum value, hours	Return to normal values, days		
AST	1954	105,000	3–4	15–28	5	++	+
LDH	1955	140,000	5–10	60–144	12	++	+
CK total enzyme activity	1960	83,000	3–9	10–20	3	++	+
CK-MB activity	1972	83,000	3–8	10–20	3	++	++
Myoglobin	1978	17,800	1–3	4–7	1–1.5	+++	+
CK-MB mass	1985	83,000	3–12	12–18	2–3	+++	+++
cTnI	1987	23,900	3–7	10–20	10	++++	++++
cTnT	1989	37,000	3–8	15–120	14	++++	++++

AST, aspartate aminotransferase; LDH, lactate dehydrogenase; CK, creatine kinase; CK-MB, creatine kinase MB isoenzyme; cTnI, cardiac troponin I; cTnT, cardiac troponin T.

Heart failure (HF) is a complex clinical syndrome resulting from any structural or functional impairment of ventricular filling or ejection of blood, including disorders of the pericardium, myocardium, endocardium, heart valves, great vessels, or certain metabolic abnormalities (Colucci, 2023). Most patients with HF have symptoms due to impaired left ventricular (LV) myocardial function (Colucci & Dunlay, 2022; Yancy et al., 2013). The most common symptoms of HF are dyspnea and fatigue, which may limit exercise tolerance and fluid retention. Some patients have exercise intolerance but little evidence of fluid retention, whereas others complain primarily of edema, dyspnea, or fatigue (Colucci & Dunlay, 2022). Heart failure is often a progressive condition, beginning with predisposing factors and leading to the development and worsening of clinical illness (Colucci, 2021; Colucci & Dunlay, 2022).

Lactate Dehydrogenase (LDH, also known as LD)

Lactate dehydrogenase is a cytoplasmic enzyme present in many different tissues, such as skeletal muscle, liver, heart, kidney, and red blood cells. Five isoenzymes have been identified by gel electrophoresis and other techniques (Marshall et al., 1991). The heart isoenzymes, LD1 and LD2, have activity increases in blood five to ten hours after MI symptoms onset and remains elevated for up to ten days (Danese & Montagnana, 2016). LD has poor specificity for cardiac tissue and is generally not recommended as a biomarker for the diagnosis of MI (Amsterdam et al., 2014; Jaffe & Morrow, 2021).

Myoglobin

Myoglobin is an oxygen-binding, cytoplasmic, heme protein. It is one of the first cardiac biomarkers measurable in the serum that appears between one and three hours after MI symptoms onset. Myoglobin is present in skeletal and cardiac muscles and is cleared by the kidneys (Vaidya, 1994). Its clinical utility is limited by its poor specificity. The main reason of using myoglobin in a clinical setting was its sensitivity for MI (Danese & Montagnana, 2016); but with appearance of sensitive troponin assays, myoglobin use offers little advantage for the diagnosis of MI (Eggers et al., 2004; Kavsak et al., 2007). Currently, there are no recommendations for myoglobin to be used in the diagnosis of MI (Amsterdam et al., 2014), and its use as cardiac biomarker is discouraged (Amsterdam et al., 2014; Jaffe & Morrow, 2021).

Creatine Kinase (CK) Isoenzymes and Isoform MB (CKMB)

The cytosolic enzyme creatine kinase (CK), formerly known as creatine phosphokinase (Danese & Montagnana, 2016), is present as three cytosolic isoenzymes and one mitochondrial isoenzyme. These isoenzymes are dimers of M (muscle) and B (brain) chains that exist in three combinations: MM, MB and BB (Bessman & Carpenter, 1985). The CKMM is predominant in both heart and skeletal muscle, but CKMB is more specific for the myocardium. The total CK activity could be detected in blood 3-9 hours after MI, but it reaches the maximum levels in blood in 10-20 hours and returns to normal in about 72 hours (Penttila et al., 2000). The measurement of total CK activity is not specific to MI because it also increases in liver, biliary tract, kidneys, and skeletal muscle disease, and its measurement is problematic in older individuals with lower muscle mass (Dillon et al., 1982; Heller et al., 1983; Yusuf et al., 1987). CKMB mass (CKMB protein concentration measurements) was once the cardiac biomarker of the choice that replaced CK, CKMB activity, AST, and LDH (Danese & Montagnana, 2016). However, with arrival of cardiac troponin assays, the use of CKMB became less popular. Some clinicians advocate for the use of CKMB for diagnosis and prognosis of MI, but cardiac troponins have shown either equally reliable or superior results compared to CKMB; consequently, troponin is the recommended test for MI diagnosis now (Amsterdam et al., 2014; Jaffe & Morrow, 2021).

Engel and Rockson (2020) studied the use of CK-MB in early diagnosis of myocardial infarction within the first nine hours of the hospital stay. The authors studied 528 patient charts of patients who had complained of chest pain. An enzymatic diagnosis was assigned if CK-MB exceeded the normal values. The diagnosis of each patient before nine hours (early diagnosis) was compared to the ultimate diagnosis at 14-24 hours (final diagnosis). Of the 528 patients, 195 (36.9%) had an early MI diagnosis within nine hours and 190 patients (97.4%) of these did have an ultimate diagnosis of MI. Therefore, the authors conclude that "standard CK-MB measurements within 9 hours of arrival provided an accurate clinical assessment in > 99% of the cases" (Engel & Rockson, 2020).

Troponins

The regulatory protein troponin in the troponin complex is composed of three isoforms. Troponin C (TnC) is responsible for calcium binding and has no role to play as a cardiac biomarker. Troponin I (TnI) and Troponin T (TnT) are responsible for inhibition of ATPase activity and tropomyosin binding, respectively (Greaser & Gergely, 1971). Contrary to all previously used cardiac biomarkers, cardiac troponins have high specificity for cardiac tissue. The cardiac troponins have a specific pattern of expression because they have different amino sequences encoded by different genes for skeletal and cardiac muscles. Cardiac TnI has an additional 31-amino acid residue compared to skeletal muscle. This protein is not expressed in normal, regenerating, or diseased skeletal muscle from human or animal origin (Bodor et al., 1995). Cardiac TnT has an additional 11-amino acid residue, but this protein was also found in regenerating rat skeletal muscle, during human fetal development, and in diseased human

skeletal muscle (Anderson et al., 1991; Bodor et al., 1997; Saggin et al., 1990). In addition, cardiac TnT was also found in skeletal muscle specimens from patients with muscular dystrophy, polymyositis, and chronic renal disease (Bodor et al., 1997; McLaurin et al., 1997).

Neumann et al. (2019) evaluated high-sensitivity troponin (troponin I and T)'s ability to predict myocardial infarction and subsequent 30-day outcomes. The authors developed a risk assessment tool based on patients presenting to the emergency department with "symptoms suggestive of myocardial infarction." Concentrations of troponin I or T were measured at presentation and after early or late serial sampling. Cutoffs were then determined to create cutoffs for risk assessment. Among the 22651 patients (9604 in derivation cohort, 13047 in validation cohort), the total prevalence of myocardial infarction was 15.3%. The authors found that "lower high-sensitivity troponin concentrations at presentation and smaller absolute changes during serial sampling were associated with a lower likelihood of myocardial infarction and a lower short-term risk of cardiovascular events" (Neumann et al., 2019).

Anand et al. (2019) evaluated the adoption rate of the universal definition of myocardial infarction and the corresponding recommendations. A total of 1902 medical centers over 23 countries were surveyed, and the authors obtained answers regarding the primary biomarker, diagnostic thresholds, and clinical pathways used to identify myocardial infarction. The authors found that cardiac troponin was the primary biomarker used at 96% of surveyed sites, with 41% of these sites using high-sensitivity troponins. The sites using high-sensitivity assays were also more likely to use serial sampling (91% vs 78% using "contemporary" sensitivity troponin) and the 99% percentile diagnostic threshold (74% vs 66%). Use of creatine kinase-MB (CKMB) was "very limited" outside of Latin America (Anand et al., 2019).

In addition, other cardiac biomarkers, such as heart-type fatty acid binding protein (H-FABP) and copeptin, have been reported in the scientific literature. However, they are not commonly used in clinical settings (Jaffe & Morrow, 2021).

Boeddinghaus et al. (2020) compared the diagnostic accuracy of high-sensitivity cardiac troponin (hs-cTn) TriageTrue assay in patients with suspected myocardial infarction (MI) with other laboratory assays including f hs-cTnTElecsys assay and hs-cTnI-Architect assay. A total of 1,261 patients with patients suggestive of MI were enrolled in the study. The TriageTrue assay ruled out patients with troponin I concentration < 3 ng/l and classified these patients as low risk of MI and ruled in patients with a troponin I concentration > 60 ng/l. Out of the 1,261 patients enrolled in the study, 178 were diagnosed with MI based on troponin I levels of > 60 ng/l using the TriageTrue assay. TriageTrue troponin I concentrations were higher in patients with MI than in patients with other final diagnoses. Other diagnosis included unstable angina in 13 of 1,261 (9%), tachyarrhythmia, Takotsubo syndrome, heart failure, or myocarditis in 208 patients (17%), and noncardiac symptoms in 714 patients (57%). The AUC of the TriageTrue assay was 0.95, the hs-cTnT-Elecsys assay AUC was 0.93, and hs-cTnI-Architect assay AUC was 0.92. The TriageTrue algorithm allowed providers to make a triage decision after one hour in 401 of 545 patients. The efficacy for rule-out or rule-in was 43% for the TriageTrue, 25% in f hs-cTnTElecsys, and 22% in hs-cTnI-Architect. Ruled-out patients had cumulative event rates of 0% at 30 days and 1.6% at two years. Overall, the authors conclude that "POC-hs-cTnI-TriageTrue assay provides high diagnostic accuracy in patients with suspected MI with a clinical performance that is at least comparable to that of best-validated central laboratory assays" (Boeddinghaus et al., 2020).

Heart-type fatty acid binding protein (H-FABP)

Heart-type fatty acid binding protein, a small cytoplasmic protein present in cardiomyocytes, is believed to have a function in myocardial lipid homeostasis (Glatz & van der Vusse, 1990). Because of its small

size, this protein appears in the blood after MI almost as early as myoglobin, but it has better specificity than myoglobin for cardiac tissue (Van Nieuwenhoven et al., 1995). Seino et al compared the use of H-FABP with rapid troponin in 371 patients with acute chest pain (Seino et al., 2003). Their study demonstrated that H-FABP had significantly higher sensitivity (89%) than troponin T (22%) and myoglobin (38%), but it has lower specificity (52%) than troponin (94%). Other studies were performed to compare H-FABP to troponins; however, they were unable to demonstrate superior results compared to troponins. H-FABP is not encouraged for assessment of MI as troponins are generally superior (Jaffe & Morrow, 2021).

In a prospective, cross-sectional study, Nguyen et al. (2020) studied the diagnostic utility of H-FABP in the early diagnosis of acute MI in comparison with troponin I and CK-MB. 216 patients enrolled in the study with 179 of those diagnosed with acute MI. H-FABP, CK-MB, and troponin I levels were compared. H-FABP reached its highest concentration in 6-12 hours after symptoms of chest pain, with a mean value of 169 ng/mL in acute MI patients. The cut-off value was 5.7 ng/ml with 90.5% sensitivity and 100% specificity. The combination of H-FABP, CK-MB and troponin I together had the highest sensitivity of 97.2%. The AUC of H-FABP was observed to be 0.99, which was higher than CK-MB (0.92) and troponin I (0.86). The authors conclude that "H-FABP can be used as a reliable diagnostic cardiac biomarker in the early detection of AMI for patients who came to the emergency room within 12h of onset of chest pain" (Nguyen et al., 2020).

Copeptin

Copeptin is the 39 amino acid C-terminal fragment cleaved from pro-arginine vasopressin (AVP). After MI, copeptin levels increase rapidly and decline over the next two to five days (Khan et al., 2007). In the Copeptin Helps in the Early Detection of Patients With Acute Myocardial Infarction (CHOPIN) 16-site study involving 1,967 patients presenting within six hours of pain onset, copeptin was shown to have a potential value in ruling out MI with a negative predictive value greater than 99% when combined with TnI measurements (Maisel et al., 2013). The Advantageous Predictors of Acute Coronary Syndrome Evaluation (APACE) multicenter study, involving 1,439 patients presenting with MI symptoms, demonstrated no benefit in using copeptin as a an early rule-out cardiac biomarker for MI (Hillinger et al., 2015). Copeptin is not encouraged for assessment of MI as troponins are generally superior (Jaffe & Morrow, 2021).

ST2

Part of the interleukin-1 receptor family with two isoforms, ST2 has two isoforms: soluble ST2 (sST2) and ST2L. ST2 is the receptor of the IL-33 cytokine that can be secreted by living cells in response to cellular stress and mechanical strain. IL-33 binds the receptor complex of ST2L and IL-1R accessory protein and reduces myocardial fibrosis, prevents cardiomyocyte hypertrophy, reduces apoptosis, and improves myocardial function. The cardioprotective effects of IL-33 are specifically through the ST2L receptor. However, sST2 may also bind IL-33, blocking the interaction between IL-33/ST2L. This eliminates the cardioprotective effects of the IL-33/ST2L interaction (Pascual-Figal & Januzzi, 2015). Experimentally, this leads to cardiac hypertrophy, fibrosis, and ventricular dysfunction (Januzzi et al., 2015).

One of the main proprietary tests used to assess ST2 levels is the Presage Assay by Critical Diagnostics. This assay was approved by the FDA on December 9, 2011. According to the FDA, "The Critical Diagnostics Presage® ST2 Assay kit is an in vitro diagnostic device that quantitatively measures ST2 in serum or plasma by enzyme-linked immunosorbent assay (ELISA) in a microtiter plate format. The Presage® ST2 Assay is indicated to be used in conjunction with clinical evaluation as an aid in assessing

the prognosis of patients diagnosed with chronic heart failure." The manufacturer claims a measuring range of 3.1 ng/mL of soluble ST2 to 200 ng/mL, and the data based on 1100 samples supports this claim. These 1100 samples found coefficient of variation of under 5%, a linear curve, and a r^2=0.99 (FDA, 2011).

By-type Natriuretic Peptide (BNP)

B-type natriuretic peptide (BNP) plays a role in salt and water management as well as pressure regulation within the natriuretic peptide system. When the prohormone proBNP is cleaved, it produces BNP and N-terminal pro-BNP; BNP is released mostly from the left ventricle in the heart. An increase in the release of BNP may be indicative of heart failure and rapid measurement can establish or exclude the diagnosis of heart failure in patients with acute dyspnea.

A number of clinical assays are available for plasma BNP. These range from rapid point-of-care tests to lab tests that provide precise values for BNP. An NT-proBNP concentration greater than 900 pm/mL is "roughly" the same as a BNP concentration that is greater than 100 pg/mL (Wilson S Colucci, 2023).

Natriuretic peptide biomarkers should be measured in patients who present with dyspnea to diagnose heart failure, but these biomarkers must be considered as part of a complete patient evaluation and not used in isolation. For prognosis, natriuretic peptide biomarkers can be used in patients with chronic HF and used when patients are admitted to the hospital with acutely decompensated HF. Lastly, there may be value in measuring natriuretic peptide biomarkers predischarge from the hospital (Wilson S Colucci, 2023).

N-terminal pro-B-type Natriuretic Peptide (NT-proBNP)

Measurement of NT-proBNP is of value in diagnosis and prognosis of heart failure and other cardiovascular diseases. Studies show that the accuracy of diagnosing heart failure across various settings improves with measurement of NT-proBNP values. Like BNP, NT-proBNP is helpful when used with patients presenting with dyspnea. The "optimal" measurement value for differentiating between heart failure and other causes of dyspnea varies with patient age.

BNP and N-terminal pro-BNP both fall in concentration after effective therapeutic treatment of chronic heart failure, which means that serial measurements have shown some promise in therapeutic management. However the effectiveness and use of serial BNP measurements in monitoring patient response to acute heart failure treatment is still under investigation (Wilson S Colucci, 2023).

Proprietary Testing

Proprietary tests for various biomarkers are available in several clinical settings. Platforms including Roche's "CARDIAC Trop T Sensitive test" and Responsebio's battery of cardiac tests emphasize their speed (on the scale of minutes) and versatility (ResponseBio, 2023; Roche, 2023).

No single diagnostic test for HF exists because it is largely a clinical diagnosis based on a careful history and physical examination. However, biomarkers of cardiovascular diseases have been developed for diagnosis and prognosis, and the use of several biomarkers is now considered the standard of care. ST2 is a marker of cardiomyocyte stress and fibrosis that adds additional value to natriuretic peptides, resulting in a risk stratification of patients with a wide spectrum of cardiovascular diseases (Bayes-Genis et al., 2015).

Clinical Utility and Validity

Jeong et al. (2020) studied the diagnostic value of copeptin for early diagnosis of acute MI in comparison with troponin I and CK-MB. 271 patients complaining of chest pain within 6 hours of onset were studied within the emergency department. The diagnostic performance of copeptin, troponin I, and CK-MB was compared by assessing the AUC and ROC curve analysis. After comparing AUC, copeptin had a significantly better diagnosis value than troponin I in patients with chest pain within two hours of onset. In addition, troponin I and copeptin together had better diagnostic performance than CK-MB and troponin I combination. Overall, the authors conclude that "the combination of troponin I and copeptin improves AMI diagnostic performance in patients with early-onset chest pain in an ED setting" (Jeong et al., 2020).

Ky et al. (2011) conducted a multi-center prospective study to evaluate whether plasma ST2 levels predict adverse outcomes in 1,141 chronic heart failure outpatients. Patients in the highest ST2 tertile (ST2 > 36.3 ng/mL) had a "markedly increased" risk (hazard ratio 3.2) of adverse outcomes compared to the lowest tertile ≤22.3 ng/mL). The investigators concluded that "ST2 is a potent marker of risk in chronic heart failure and when used in combination with NT-proBNP offers moderate improvement in assessing prognosis beyond clinical risk scores" (Ky et al., 2011).

Wang et al. (2012) studied the prognostic value of three novel biomarkers induced by cardiovascular stress. The investigators measured sST2, growth differentiation factor-15, and high-sensitivity troponin I in 3,428 participants in the Framingham Heart Study. Multivariable-adjusted proportional hazards models were performed to assess the individual and combined ability of the biomarkers to predict adverse outcomes. The three new biomarkers were associated with death, major cardiovascular events, and heart failure, but not with coronary events. The investigators concluded that the findings demonstrated the prognostic value of the newer biomarkers in apparently healthy individuals (Wang et al., 2012).

Wijk et al. (2014) provided a follow-up on the largest study of long-term results of intensified NT-proBNP-guided versus symptom-guided management of elderly patients with heart failure. The TIME-CHF study randomized 499 patients with heart failure that were ages 60 and older with left ventricular ejection fraction; patients were provided either guided NT-proBNP treatment or symptom-guided therapy over a period of 18 months. The results of the study showed "NT-proBNP–guided therapy did not improve the primary end point compared with symptom-guided therapy but did improve HF hospitalization-free survival" (Wijk et al., 2014).

Wang et al. (2018) investigated the possibility of using sST2 as a biomarker to distinguish between acute aortic dissection and other causes of acute chest pain. Using an R&D Systems assay to measure plasma concentrations of sST2 in 1360 patients with a cutoff of 34.6 ng/mL, the researchers found that "sST2 had a sensitivity of 99.1%, specificity of 84.9%, positive predictive value of 68.7%, negative predictive value of 99.7%, positive likelihood ratio of 6.6, and negative likelihood ratio of 0.01." Additionally, within 24 hours of symptom onset, sST2 levels were higher in those with acute aortic dissection in comparison to those with acute myocardial infarction or pulmonary embolism. sST2 was also superior in overall diagnostic performance to D-dimer and troponin I using the area under receiver operating characteristic curves.

Januzzi et al. (2013) conducted a retrospective study to assess sST2 as a prognostic marker after orthotopic heart transplantation (OHT) and as a test to predict acute cellular rejection. sST2 concentrations were measured in 241 patients following OHT. Elevated sST2 was associated with cellular

rejection, with highest rates of cellular rejection in the 4th sST2 quartile. No significant association between sST2 and antibody-mediated rejection or allograft vasculopathy was found. A sST2 level of ≥ 30 ng/mL was found to independently predict death over the 7-year follow-up with a hazard ratio of 2.1. The investigators concluded that sST2 levels are associated with the presence of cellular rejection and predict long-term mortality following OHT (Januzzi et al., 2013).

Boman et al. (2018) assessed the prognostic value of ST2 on cardiovascular mortality. 159 patients were evaluated, but ST2 was not found to be significantly associated with cardiovascular mortality or all-cause mortality. Furthermore, no significant interaction of ST2 and N-terminal pro-hormone of brain natriuretic peptide /N-terminal pro-B-type natriuretic peptide was found (Boman et al., 2018).

Dimitropoulos et al. (2020) investigated the association of soluble suppression of tumorigenesis-2 (sST2) with endothelial function in patients with ischemic heart failure. A total of 143 patients with "table HF of ischemic etiology and reduced left ventricular ejection fraction (LVEF)" were included along with 77 controls. The authors found an increased level of sST2 in HF patients compared to controls (15.8 ng/mL compared to 12.5 ng/mL). Within the HF group, there was no association of LVEF with sST2. Overall, sST2 levels were found to be increased and associated with functional capacity in "patients with chronic HF of ischemic etiology." Finally, the authors found an inverse association between flow-mediated dilation and sST2 levels, which the authors stated "highlight[ed] the interplay between the dysfunctional endothelium and HF pathophysiologic mechanisms" (Dimitropoulos et al., 2020).

Hou et al. (2020) aimed to investigate the association between sST2 levels and clinical outcomes of high-risk heart failure. The primary endpoint was defined as all-cause mortality. A total of 150 patients were included; all-cause mortality occurred in 16 of the patients over the course follow-up. The authors found that all-cause mortality increased significantly above 34.98846 ng/mL by a factor of 16% to 5.33%. After adjusting the model for certain co-factors (age, gender, et al.), and after adding NT-proBNP, "the risk of all-cause death was increased by 2.5% and 1.9%, respectively, per ng/ml of sST2." The authors identified the best sST2 cut-off for predicting all-cause mortality to be 43.42671 ng/ml, with an area under the curve of 0.72, sensitivity of 0.69, and specificity of 0.69. Risk of all-cause mortality was found to be 21.2% above this cutoff and 5.1% below it, with a corresponding hazard ratio of 3.30. The authors concluded that "Patients with sST2 levels more than 43.42671 ng/ml even after ICD implantation should therefore be monitored carefully" (Hou et al., 2020).

Guidelines and Recommendations

2018 ESC/ACC/AHA/WHF Fourth Universal Definition of Myocardial Infarction

Both cTnI and cTnT are recommended for evaluation of myocardial injury, and high sensitivity cTn assays are recommended for routine clinical use. An acute MI is designated when a rising/falling pattern is seen with cTn levels and if there is at least one measurement greater than the 99th percentile of the upper reference limit (URL) (Jaffe et al., 2018).

CKMB is considered less sensitive and specific than either troponin. However, in the absence of a cTn assay, CK-MB is considered the best alternative. A measurement of CK-MB above the 99th percentile of the URL should be "designated as the decision level for the diagnosis of MI." Sex-specific CK-MB values should be used (Jaffe et al., 2018).

In the 2019 AHA guideline discussing the "Contemporary Diagnosis and Management of Patients With Myocardial Infarction in the Absence of Obstructive Coronary Artery Disease [MINOCA]", the AHA notes that the diagnostic criteria of MINOCA follows the "Fourth Universal Definition of Myocardial Infarction"

above, specifically the rise or fall of cardiac troponin levels with at least one value above the 99th percentile of the reference limit. The guideline considers this definition "fundamental" to identifying and defining MINOCA (Tamis-Holland Jacqueline et al., 2019).

2014 AHA/ACC Guideline for the Management of Patients with Non-ST-Elevation Acute Coronary Syndromes (NSTE-ACS)

The American College of Cardiology (ACC) and the American Heart Association (AHA) have developed clinical practice guidelines to provide recommendations applicable to patients with or at risk of developing cardiovascular disease and to provide guidance to clinicians on optimal management of patients with NSTE-ACS. In their comprehensive document, the AHA/ACC panel has provided recommendations for initial evaluation and management of patients presenting with ACS symptoms, for the early hospital care, myocardial revascularization, late hospital care, hospital discharge and posthospital discharge care, special patient groups and quality of care and outcomes for ACS. The Task Force recommended to stratify patients with suspected ACS based on the likelihood of ACS and those with high-risk features should be referred immediately to the emergency department (ED). They have provided specific recommendations for the use of cardiac biomarkers in the diagnosis and prognosis of MI. They specifically recommended using troponin (troponin I or T when contemporary assay is used) for the diagnosis of MI. According to AHA/ACC guidelines, the cardiac troponin is recommended and should be measured at presentation and three to six hours after symptom onset in all patients who present with ACS symptoms. The panelists recommended identifying rising and/or falling pattern of troponin. In addition, they recommended measuring troponin levels beyond six hours after symptom onset in patients with normal troponins on serial examination when ECG changes and/or clinical presentation suggests ACS. If the onset of symptoms is not clearly identified, they recommended using the time of presentation as the time of onset for measuring troponin. The AHA/ACC guideline clearly highlighted that CKMB, or myoglobin should not be used for the diagnosis of ACS. All recommendations for the use of cardiac biomarkers in the diagnosis of MI were level A evidence.

The AHA/ACC guideline considered all recommendations in the use of cardiac biomarkers for ACS prognosis as level of evidence B. They considered the presence and magnitude of troponin elevations useful for short- and long-term prognosis. The re-measurement of troponin once on day three or four in patients with MI was considered reasonable to estimate the infarct size and dynamics of necrosis. Finally, they considered the use of B-type natriuretic peptide to be reasonable for additional prognostic information.

The recommendations for the use of cardiac biomarkers in the diagnosis and prognosis of MI was well summarized in Table from 2014 AHA/ACC guidelines p.2655 (Amsterdam et al., 2014):

TABLE 5 Summary of Recommendations for Cardiac Biomarkers and the Universal Definition of MI

Recommendations	COR	LOE
Diagnosis		
Measure cardiac-specific troponin (troponin I or T) at presentation and 3–6 h after symptom onset in all patients with suspected ACS to identify pattern of values	I	A
Obtain additional troponin levels beyond 6 h in patients with initial normal serial troponins with electrocardiographic changes and/or intermediate/high risk clinical features	I	A
Consider time of presentation the time of onset with ambiguous symptom onset for assessing troponin values	I	A
With contemporary troponin assays, CK-MB and myoglobin are not useful for diagnosis of ACS	III: No Benefit	A
Prognosis		
Troponin elevations are useful for short- and long-term prognosis	I	B
Remeasurement of troponin value once on d 3 or 4 in patients with MI may be reasonable as an index of infarct size and dynamics of necrosis	IIb	B
BNP may be reasonable for additional prognostic information	IIb	B

ACS indicates acute coronary syndromes; BNP, B-type natriuretic peptide; CK-MB, creatine kinase myocardial isoenzyme; COR, Class of Recommendation; LOE, Level of Evidence; and MI, myocardial infarction.

2013 (published 2014) Society for Cardiovascular Angiography and Interventions (SCAI)

In their expert consensus document titled "Consideration of a New Definition of Clinically Relevant Myocardial Infarction After Coronary Revascularization," the SCAI expert panel introduced a new definition of clinically relevant MI after coronary revascularization percutaneous coronary intervention (PCI) or coronary artery bypass grafting (CABG). In their definition of clinically relevant MI after both PCI and CABG procedures, authors gave recommendations according to three different types of clinical presentation. In the first case, when patient has a normal CKMB baseline: "The peak CK-MB measured within 48 hours of the procedure rises to $\geq$10x the local laboratory ULN, or to $\geq$5x ULN with new pathologic Q-waves in $\geq$2 contiguous leads or new persistent LBBB, OR in the absence of CK-MB measurements and a normal baseline cTn, a cTn (I or T) level measured within 48 hours of the PCI rises to $\geq$70x the local laboratory ULN, or $\geq$35x ULN with new pathologic Q-waves in $\geq$2 contiguous leads or new persistent LBBB." In the case when patients have elevated baseline CKMB (or cTn) with stable of falling biomarkers levels, they issued the following recommendation: "The CK-MB (or cTn) rises by an absolute increment equal to those levels recommended above from the most recent pre-procedure level." And, in patients with elevated CKMB (or cTn), but without stable or falling biomarkers level, the recommendation was: "The CK-MB (or cTn) rises by an absolute increment equal to those levels recommended above plus new ST-segment elevation or depression plus signs consistent with a clinically relevant MI, such as new onset or worsening heart failure or sustained hypotension." The authors have expressed preference to use CKMB instead of cTn, but they have included cTn in their definition if CKMB was not available (Moussa et al., 2013).

2015 AHA Guidelines Update for Cardiopulmonary Resuscitation and Emergency Cardiovascular Care

In their review of previously issued guidelines, the expert panel introduced new recommendations for diagnostic interventions in ACS regarding cardiac biomarkers. They still recommended to use Troponin in following situations: "We recommend against using hs-cTnT and cTnI alone measured at 0 and 2 hours (without performing clinical risk stratification) to identify patients at low risk for ACS (Class III: Harm, LOE B-NR). We recommend that hs-cTnI measurements that are less than the 99th percentile, measured at 0 and 2 hours, may be used together with low-risk stratification (TIMI score of 0 or 1 or low risk per Vancouver rule) to predict a less than 1% chance of 30-day MACE (Class IIa, LOE B-NR). We recommend that negative cTnI or cTnT measurements at 0 and between 3 and 6 hours may be used together with very low-risk stratification (TIMI score of 0, low-risk score per Vancouver rule, North American Chest Pain score of 0 and age less than 50 years, or low-risk HEART score) to predict a less than 1% chance of 30-day MACE (Class IIa, LOE B-NR)." They did not express a preference in cardiac biomarkers to use, nor did they gave any recommendations regarding CKMB (O'Connor Robert et al., 2015).

American College of Cardiology/American Heart Association/Heart Failure Society of America (ACC/AHA/HFSA)

In 2017, the ACC/AHA/HFSA included information on BNP and NT-proBNP measurement for establishing prognosis or disease severity in chronic HF. Their recommendations:

- For prevention:
  - "Class IIa recommendation (Level of Evidence: B-R) for utilizing natriuretic peptide biomarker-based screening for those at risk of developing HF, followed by team-based care including a cardiovascular specialist optimizing guideline-directed medical therapy (GDMT), to prevent the development of left ventricular dysfunction (systolic or diastolic) or new-onset HF.z"
- For diagnosis:
  - "Class I recommendation (Level of Evidence: A) for measurement of natriuretic peptide biomarkers in patients presenting with dyspnea, to support a diagnosis or exclusion of HF."
- For prognosis or added risk stratification:
  - "Class I recommendation (Level of Evidence: A) for measurement of B-type natriuretic peptide (BNP) or N-terminal (NT)-proBNP for establishing prognosis or disease severity in chronic HF."
  - "Class I recommendation (Level of Evidence: A) for measurement of baseline natriuretic peptide biomarkers and/or cardiac troponin on admission to the hospital to establish a prognosis in acutely decompensated HF."
  - "Class IIa recommendation (Level of Evidence: B-NR) for measurement of a predischarge natriuretic peptide level during a HF hospitalization, to establish a post-discharge prognosis."
  - "Class IIa recommendation (Level of Evidence: B-NR) for measurement of other clinically available tests, such as biomarkers of myocardial injury or fibrosis, in patients with chronic HF for additive risk stratification (Yancy et al., 2017)."

The full ACC/AHA article does not appear to support a standard of care that includes measuring BNP/NT-BNP for purposes of serial monitoring or therapeutic management, noting that "Because of the absence of clear and consistent evidence for improvement in mortality and cardiovascular outcomes, there are insufficient data to inform specific guideline recommendations related to natriuretic peptide-guided therapy or serial measurements of BNP or NT-proBNP levels for the purpose of reducing hospitalization or deaths in the present document" (Yancy et al., 2017).

In 2022, the ACC/AHA/HFSA updated their 2017 guideline on the management of heart failure. Regarding BNP and NT-proBNP assays, the authors emphasize that both tests can be used to establish

the presence and severity of heart failure. However, they caveat that diagnostic sensitivity is impacted when a patient Is overweight – patients who are obese sometimes measure as having low levels of BNP and NT-proBNP.

Additional points of emphasis include:

- "A substantial evidence base supports the use of natriuretic peptide biomarkers for excluding HF as a cause of symptoms in ambulatory and emergency department settings."
- "Although a reduction in BNP and NT-pro-BNP has been associated with better outcomes, the evidence for treatment guidance using serial BNP or NT-proBNP measurements remains insufficient."
- "A widening array of biomarkers including markers of myocardial injury, inflammation, oxidative stress, vascular dysfunction, and matrix remodeling have been shown to provide incremental prognostic information over natriuretic peptides but remain without evidence of an incremental management benefit."

The recommendations for the use of biomarkers was summarized in the table provided by the ACC/AHA/HFSA (Heidenreich, 2022):

Recommendations for Use of Biomarkers for Prevention, Initial Diagnosis, and Risk Stratification
Referenced studies that support the recommendations are summarized in the Online Data Supplements.

COR	LOE	RECOMMENDATIONS
1	A	1. In patients presenting with dyspnea, measurement of B-type natriuretic peptide (BNP) or N-terminal prohormone of B-type natriuretic peptide (NT-proBNP) is useful to support a diagnosis or exclusion of HF (1-12).
1	A	2. In patients with chronic HF, measurements of BNP or NT-proBNP levels are recommended for risk stratification (11,13-29).
1	A	3. In patients hospitalized for HF, measurement of BNP or NT-proBNP levels at admission is recommended to establish prognosis (11,13-19).
2a	B-R	4. In patients at risk of developing HF, BNP or NT-proBNP–based screening followed by team-based care, including a cardiovascular specialist, can be useful to prevent the development of LV dysfunction or new-onset HF (30,31).
2a	B-NR	5. In patients hospitalized for HF, a predischarge BNP or NT-proBNP level can be useful to inform the trajectory of the patient and establish a postdischarge prognosis (14,17,20-29).

American Heart Association (AHA)

The AHA notes sST2 as an "emerging" biomarker that supports diagnosis of HF with preserved ejection fraction, a biomarker that may predict mortality and HF events, and a biomarker that correlates with left ventricular end-diastolic pressure. The AHA states that sST2 has numerous advantages as a biomarker, namely its concentration being unaffected by BMI, age, or renal function. SST2 is stated to correlate with HF prognosis as well. Overall, AHA states that out of the newer biomarkers (SST2, ST2, Gal-3, and GDF-15), "most appeal is driven by sST2" (Chow et al., 2017).

A Scientific Statement published in 2019 also considered ST2 as the most "promising clinically", but also mentioned the limitations in consistency and utility in most inflammatory mediators. The Statement

notes several clinical studies focusing on sST2 that are in progress as of March 24, 2020 (Cresci et al., 2019).

European Society of Cardiology (ESC)

The ESC notes measurement of cardiac troponins as "mandatory" in all patients with suspected non-ST-elevation acute coronary syndromes. The guidelines assert that cardiac troponins are more sensitive and specific biomarkers of cardiomyocyte injury than CK, CKMB, and myoglobin. However, if troponin measurement is not possible, measurement of copeptin is recommended.

The ESC also acknowledges the natriuretic peptides (B-type natriuretic peptide, N-terminal pro-B-type natriuretic peptide and midregional pro-A-type natriuretic peptide) as providing useful prognostic information along with the troponins. The ESC mentions other biomarkers such as midregional pro-adrenomedullin, growth differentiation factor 15 and copeptin, but they cannot recommend them at this time as their added value in risk assessment seems "marginal" (Gencer et al., 2016).

The 2019 ESC guidelines focusing on chronic coronary syndromes states that for "clinical suspicion of coronary artery disease instability…management should follow the Guidelines for ACS without persistent ST-segment elevation", which is discussed above (Knuuti et al., 2019).

The 2020 ESC guidelines focus on diagnosis of acute coronary syndrome. Regarding MI, they recommend that "the routine use of copeptin as an additional biomarker for the early rule-out of MI should be considered where hs-cTn assays are not available." In addition, "CK-MB shows a more rapid decline after MI and may provide added value for detection of early reinfarction" (Collet et al., 2021).

In their 2016 guidelines on acute and chronic heart failure, the ESC states that "although there is extensive research on biomarkers in HF (e.g. ST2, galectin 3, copeptin, adrenomedullin), there is no definite evidence to recommend them for clinical practice" (Ponikowski et al., 2016).

However, in a 2021 update, ESC (with special contribution from the Heart Failure Association) lists the key elements for HF and CMP diagnostic workups, and among the laboratory exams recommended under Table 26 for the "Initial diagnostic assessment in patients with suspected cardiomyopathy", they include the use of ST2:

"**Laboratory exams** including cardiac and muscular enzymes, liver and renal function, haemoglobin, white blood cell count (including differential white blood cell count to detect eosinophilia), natriuretic peptides, thyroid function tests, iron status, and markers of systemic auto-immune disease (hsCRP, anti-nuclear antibodies, soluble IL-2 receptor)" (McDonagh et al., 2021)

Heart Failure Association of the European Society of Cardiology

The Heart Failure Association of the European Society of Cardiology published a position statement on Advanced Heart Failure which states: "Post-transplant patients should undergo a pre-defined regimen of graft biopsies, titration of immunosuppressive and other therapies, rejection monitoring, assessment for infections, transplant coronary artery disease and/or cardiac allograft vasculopathy, immunosuppression side effects, and other potential complications including neoplasia, and co-morbidities that require comprehensive treatment." However, the guideline does not mention sST2 regarding prognosis of post-transplant patients (Crespo-Leiro et al., 2018).

ACC/AATS/AHA/ASE/ASNC/SCAI/SCCT/STS 2016 Appropriate Use Criteria for Coronary Revascularization in Patients With Acute Coronary Syndromes Guidelines

In 2016 The American College of Cardiology (ACC), Society for Cardiovascular Angiography and Interventions (SCAI), Society of Thoracic Surgeons (STS), and American Association for Thoracic Surgery (AATS), along with key specialty and subspecialty societies created an Appropriate Use Task Force with the mission to revise the appropriate use criteria (AUC) for coronary revascularization. They have used clinical scenarios to mimic patient presentations seen in everyday clinical practice and included information on symptom status, presence of clinical instability or ongoing ischemic symptoms and other characteristics. They follow 2014 AHA/ACC recommendations for the use of cardiac biomarkers (Amsterdam et al., 2014).

American Society for Clinical Pathology (ASCP)

The ASCP recommends against testing CK-MB or myoglobin to diagnose an acute myocardial infarction. Instead, they recommend testing either troponin I or T. They also assert that both troponins are specific to cardiac injury and that there is much support for relying solely on troponin (ASCP, 2015).

National Institute for Health and Care Excellence (NICE)

NICE recommends diagnosis of MI using the "detection of rise and/or fall of cardiac biomarkers values [preferably cardiac troponin (cTn)] with at least one value above the 99th percentile of the upper reference limit and at least one of the following:

- symptoms of ischaemia
- new or presumed new significant ST-segment-T wave (ST-T) changes or new left bundle branch block (LBBB)
- development of pathological Q waves in the ECG
- imaging evidence of new loss of viable myocardium or new regional wall motion abnormality
- identification of an intracoronary thrombus by angiography" (NICE, 2016).

Currently, the 2018 NICE recommendations on chronic heart failure do not mention the usage of ST2 as a marker for diagnosing chronic heart failure. Instead, they recommend to "measure N-terminal pro-B-type natriuretic peptide (NT-proBNP) in people with suspected heart failure" (NICE, 2018).

In 2020, NICE released recommendations on the use of high sensitivity troponin tests to help rule out NSTEMI earlier in those presenting to an emergency department with chest pain and suspected acute coronary syndrome. NICE recommends the use of the following assays: Access High-Sensitivity Troponin I Assay, ADVIA Centaur High-Sensitivity Cardiac Troponin-I Assay, Alinity High Sensitive Troponin-I assay, ARCHITECT STAT High Sensitive Troponin-I assay, Atellica IM High-Sensitivity Cardiac Troponin I Assay, Dimension Vista High-Sensitivity Cardiac Troponin I Assay, Dimension EXL High-Sensitivity Cardiac Troponin I Assay, Elecsys Troponin T-high sensitive assay, Elecsys Troponin T-high sensitive STAT assay, VIDAS High sensitive Troponin I assay, and VITROS High Sensitivity Troponin I Assay. NICE mentions that although the "TriageTrue test has the potential to be cost effective, its diagnostic accuracy when used on whole blood is uncertain" (NICE, 2020). Regarding use of these assays, NICE recommends using a threshold at or near the limit of detection, which varies depending on the assay used. If this sample is positive, it should not be used to rule in NSTEMI. If taking multiple samples, take a sample at initial assessment followed by a second sample taken 30 minutes to three hours after. Use 99th percentile thresholds or thresholds at or near the limit of detection of the assay (NICE, 2020).

Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
82550	Creatine kinase (CK), (CPK); total
82552	Creatine kinase (CK), (CPK); isoenzymes
82553	Creatine kinase (CK), (CPK); MB fraction only
82554	Creatine kinase (CK), (CPK); isoforms
82725	Fatty acids, nonesterified
83006	Growth stimulation expressed gene 2 (ST2, Interleukin 1 receptor like-1)
83615	Lactate dehydrogenase (LD), (LDH);
83625	Lactate dehydrogenase (LD), (LDH); isoenzymes, separation and quantitation
83874	Myoglobin
83880	Natriuretic peptide
84450	Transferase; aspartate amino (AST) (SGOT)
84484	Troponin, quantitative
84512	Troponin, qualitative
84588	Vasopressin (antidiuretic hormone, ADH)
84999	Unlisted chemistry procedure
86140	C-reactive protein

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

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Revision History

Revision Date	Summary of Changes
09/06/2023	Reviewed and Updated: Updated background, guidelines, and evidence-based scientific references. Literature review necessitated the following changes in coverage criteria: New CC3: "3) Measurement of B-type natriuretic peptide (BNP) or N-terminal proBNP (NT-proBNP) MEETS COVERAGE CRITERIA in any of the following situations: a) To diagnose heart failure in individuals presenting with dyspnea. b) To establish disease severity in individuals with chronic heart failure (up to four times per year in the outpatient setting)." CC4 edited for clarity, "for all situations" changed to "In the outpatient setting" Added CPT code 83880

Bone Turnover Markers Testing

Policy Number: AHS - G2051 – Bone Turnover Markers Testing	Prior Policy Name and Number, as applicable: • AHS - G2051 – Bone Turnover Markers for Diagnosis and Management of Osteoporosis and Diseases Associated with High Bone Turnover
Initial Presentation Date: 09/18/2015 Last Review Date: February 1, 2025	

Policy Description

Bone metabolism involves a continual, dynamic equilibrium between bone growth and resorption. Bone turnover markers (BTMs) are biochemical markers for assessment of bone formation or bone resorption. These markers may be useful in determining risk of fracture and bone loss (Rosen, 2021b).

Related Policies

Policy Number	Policy Title
AHS-G2005	Vitamin D Testing
AHS-G2164	Parathyroid Hormone, Phosphorus, Calcium, and Magnesium Testing

Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document. *Bone turnover markers are listed in Note 1.*

1) For individuals treated with bisphosphonates, measurement of bone turnover markers to assess an individual's compliance with bisphosphonate therapy or for fracture risk prediction **MEETS COVERAGE CRITERIA** at the following intervals:

a) To establish baseline levels before initiating bisphosphonate treatment

b) Every three months after initiation or change of therapy for the first year.

c) Every two years when no medication changes have occurred.

2) For individuals with osteoporosis, measurement of bone turnover markers to monitor teriparatide treatment **DOES NOT MEET COVERAGE CRITERIA**.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

3) As a diagnostic test for osteoporosis, measurement of bone turnover markers **DOES NOT MEET COVERAGE CRITERIA**.

4) In the diagnosis and management of patients with other conditions associated with high rates of bone turnover, measurement of bone turnover markers **DOES NOT MEET COVERAGE CRITERIA**.

NOTES:

Note 1: Bone turnover markers include (Rosen, 2021a, 2021b; Talwar, 2020):

1. Bone formation markers
   a. Serum bone–specific alkaline phosphatase (BSAP/BALP)
   b. Serum osteocalcin (OC)
   c. Serum type 1 procollagen (C-terminal/N-terminal): C1NP or P1NP
2. Bone resorption markers
   a. Urinary hydroxyproline (HYP)
   b. Urinary total pyridinoline (PYD)
   c. Urinary free deoxypyridinoline (DPD)
   d. Urinary or serum collagen type 1 cross-linked N-telopeptide (NTX)
   e. Urinary or serum collagen type 1 cross-linked C-telopeptide (CTX)
   f. Bone sialoprotein (BSP)
   g. Serum Tartrate-resistant acid phosphatase 5b (TRACP5b)
   h. Cathepsin K

Table of Terminology

Term	Definition
AACE	American Association of Clinical Endocrinologists
ACE	American College of Endocrinology
AFOS	Asian Federation of Osteoporosis Societies
ALP	Alkaline phosphatase
B-ALP	Bone-specific alkaline phosphatase
BAP	Bone-specific alkaline phosphatase
BMA	Bone marker assays
BMD	Bone mass density

BMD	Bone mineral density
BP	Bisphosphonates
BSAP/BALP	Bone-specific alkaline phosphatase specific to osteoblasts
BSP	Bone sialoprotein
BTMs	Bone turnover markers
C1NP or P1NP	Type 1 procollagen (C-Terminal/N-Terminal)
CKD	Chronic kidney disease
CKD-MBD	Chronic kidney disease–mineral and bone disorder
CLIA ‘88	Clinical Laboratory Improvement Amendments of 1988
CMS	Centers for Medicare and Medicaid
CTX	C-CT terminal telopeptide of type I collagen
CV	Coefficient of variation
DEXA	Dual energy x-ray absorptiometry
DPD	Deoxypyridinoline
ELISA	The enzyme-linked immunosorbent assay
ESCEO	Economic aspects of osteoporosis and osteoarthritis
FDA	Food and Drug Administration
FRAX	Fracture Risk Assessment Tool
GR	Gradient of risk
HYP	Hydroxyproline
ICTP	Serum c-terminal cross-linked telopeptide of type I collagen
IFCC	International Federation of Clinical Chemistry and Laboratory Medicine
IOF	International Osteoporosis Foundation
ISCD	International Society for Clinical Densitometry
KDIGO	Kidney disease improving global outcomes
LDTs	Laboratory-developed tests
LSC	Least significant change
NAMS	North American Menopause Society
NOGG	National Osteoporosis Guideline Group
NTX	N-telopeptide
NTX	Cross-linked n-telopeptides of type 1 collagen
OC	Osteocalcin
PDB	Paget's disease of bone
PHPT	Primary hyperparathyroidism
P1CP	Carboxy terminal propeptide
P1NP	Procollagen type I n propeptide
PTH	Parathyroid hormone
PYD	Pyridinoline
RACGP	Royal Australian College of General Practitioners
ROS	Royal Osteoporosis Society
s-CTX	Serum c-terminal cross-linking telopeptide of type I collagen
s-PINP	Serum procollagen type I N propeptide
TH	Total hip
TRACP-5b/TRAP-5B	Tartrate-resistant acid phosphatase 5b
USPSTF	United States Preventative Services Task Force

βCTX-I	Bone alkaline phosphatase for bone formation and c-terminal cross-linking telopeptide of type I collagen

Scientific Background

The resorption and reformation of bone are normally tightly regulated and coupled so that bone mass does not change. Bone disease occurs when these processes are uncoupled (Rosen, 2021a, 2021b). Biomarkers involved in the processes of resorption or formation have been proposed as measures for prediction of future bone loss, fracture risk, and more. Resorption markers include pyridinium crosslinks (PYD, DPD), C- and N-telopeptides (CTX, ICTP, NTX), tartrate-resistant acid phosphatase (TRACP) 5b, and cathepsin K, while formation markers include procollagen type I propeptides (PICP, PINP), osteocalcin, and bone-specific alkaline phosphatase (BSAP, also known as BALP) (Rosen, 2021a, 2021b).

Formation markers are characteristic of bone formation rate. PICP and PINP are carboxy- and amino-sides of the tropocollagen peptide, which is a precursor to type I collagen in bone. The serum concentration of these peptides reflects synthesis of new collagen. Osteocalcin is a component of osteoid, and BSAP is the alkaline phosphatase specific to osteoblasts. These biomarkers reflect the activity of osteoblasts. Of these markers, BSAP and PINP are considered the most clinically useful (Rosen, 2021a, 2021b).

Resorption markers are characteristic of bone resorption rate (breakdown of bone). Pyridinium crosslinks are components of bone collagen, C- and N- telopeptides are crosslinks between bone collagen molecules, TRACP is anchored to the osteoclasts that initiate bone resorption, and cathepsin K is involved in digestion of the organic matrix (Manolagas, 2022; Rosen, 2021a, 2021b). Of these markers, urinary NTX and serum CTX are considered the most clinically useful (Rosen, 2021a, 2021b).

The measurement and use of these biomarkers remain complicated. Biologic variability between and within patients is significant, as factors such as age, gender, body mass index, circadian rhythms, menstruation, smoking, time of food consumption, exercise, and more may influence the levels of BTMs (Rosen, 2021a, 2021b). Moreover, assays used to measure these biomarkers vary considerably, as both urinary and serum samples have been used. Lack of standardization has limited the use of BTMs in the clinical setting (Rosen, 2021a, 2021b).

Vitamin D supplementation has been used in the past for musculoskeletal diseases in both a prevention and treatment capacity— but data on supplementation with vitamin D and any corresponding effects on bone resorption and formation has been inconclusive. One study that investigated the effects of vitamin D supplementation on bone turnover markers such as bone-specific alkaline phosphatase (bALP), osteocalcin (OC), C-terminal telopeptide (CTX), and procollagen type 1 N-terminal propeptide (P1NP) failed to show any significant impact of vitamin D on bone turnover markers (Schwetz et al., 2017), while another study noted "a small, but significant, decrease in the bone formation marker procollagen of type 1 amino-terminal propeptide (P1NP)—in the vitamin D group as compared to the placebo group" (Jorde et al., 2019).

Analytical Validity

Eastell et al. (2000) assessed the biological variability between serum and urinary N-telopeptides of type I collagen (NTX). A total of 277 postmenopausal individuals were included, and urine and serum specimens were included to identify short-term variability. Long-term variability was determined by comparing NTX at baseline and at two months. The authors found the median short-term coefficient of

variation (CV) was 13.1% for urinary NTX and 6.3% for serum NTX. Long-term CV% was found to be 15.6% for urinary NTX and 7.5% for serum NTX. The authors also observed that to be 90% confident that a decrease in NTX after antiresorptive therapy was not caused by variability alone, a 31% decrease in urinary NTX and a 14% decrease in serum NTX are needed (Eastell et al., 2000).

Seibel et al. (2001) described the results of an international proficiency testing program for biochemical bone markers among clinical laboratories. The authors sent out two urinary and two serum pools (both normal and increased concentrations of markers) to 79 laboratories. The CVs were as follows: "serum bone-specific alkaline phosphatase (n = 47 laboratories), 16–48%; serum osteocalcin (n = 31), 16–42%; urinary free deoxypyridinoline (n = 30), 6.4–12%; urinary total deoxypyridinoline and pyridinoline (n = 29), 27–28%; urinary N-terminal cross-linked telopeptide of type I collagen (n = 10), 39%; serum C-terminal cross-linked telopeptide of type I collagen (ICTP; n = 8), 22–27%; urinary hydroxyproline (n = 13), 12%". The authors concluded that "even with identical assays and methods, results for most biochemical markers of bone turnover differ markedly among laboratories" (Seibel et al., 2001).

Schafer et al. (2010) assessed the laboratory reproducibility of urine N-telopeptide (NTX) and serum bone-specific alkaline phosphatase (BAP). The authors obtained serum and urine from five postmenopausal individuals and sent specimens to six labs over eight months. They found that "Longitudinal coefficients of variation (CVs) ranged from 5.4% to 37.6% for NTX and from 3.1% to 23.6% for BAP. Within-run CVs ranged from 1.5% to 17.2% for NTX" (Schafer et al., 2010).

Hlaing and Compston (2014) notes that "although automated platforms have substantially improved the analytical variability of bone turnover markers, reproducibility still varies substantially". The National Bone Health Alliance executed a project to standardize bone turnover marker collection procedures and reduce pre-analytical variability (Bauer et al., 2012). The results of that project and the IOF and IFCC Bone Marker Standards Working Group identification of PINP and CTX-I in blood to be the reference markers of bone turnover for the fracture risk prediction and monitoring of osteoporosis treatment (Vasikaran et al., 2011) have resulted in recommendations for standard sample handling and patient preparation (Szulc et al., 2017). Standardization and harmonization of clinical assays for bone turnover markers such as CTx and P1NP are ongoing (IFCC, 2020).

Clinical Utility and Validity

Johansson et al. (2014) performed a meta-analysis to "examine the performance characteristics of serum procollagen type I N propeptide (s-PINP) and serum C-terminal cross-linking telopeptide of type I collagen (s-CTX) in fracture risk prediction in untreated individuals in prospective cohort studies." Six studies were included. The authors identified a "significant" association between s-CTX and risk of fracture (gradient of risk [GR] = 1.18). The hazard ratio per standard deviation increase in s-PINP was found to be 1.23 and was unadjusted for bone mineral density. The association between s-CTX and fracture risk was found to be 1.23. The authors concluded that "there is a modest but significant association between BTMs and risk of future fractures" (Johansson et al., 2014).

Marques et al. (2016) "assessed whether circulating bone formation and resorption markers (BTM) were individual predictors for trabecular and cortical bone loss, periosteal expansion, and fracture risk in older adults aged 66 to 93." A total of 1069 participants were included in the study. Bone formation was assessed by serum procollagen type I N propeptide (PINP) and osteocalcin, and bone resorption was assessed by C-terminal cross-linking telopeptide of type I collagen (CTX). Inter-assay coefficients of variation were <3% for all BTM. A total of 236 participants sustained a fracture during the median

follow-up of 11.7 years. The authors found that "increase in BTM levels was associated with faster cortical and trabecular bone loss at the femoral neck and proximal femur. Higher BTM levels were positively related with periosteal expansion rate at the femoral neck in men. Markers were not associated with fracture risk" (Marques et al., 2016).

Mederle et al. (2018) investigated the correlation between bone mass density (BMD) and "serum levels of BTMs (tartrate-resistant acid phosphatase-5b [TRAP-5b]), bone-specific alkaline phosphatase (BSAP), in postmenopausal osteoporotic individuals as compared to healthy postmenopausal subjects." A total of 132 postmenopausal individuals with osteoporosis were included along with 81 healthy postmenopausal individuals. BSAP was found to have a sensitivity of 76.5% and specificity of 84.3% at a cutoff of 21.27 U/L, and TRAP-5b was found to have a sensitivity of 86.3% and specificity of 90.6% at a cutoff of 3.45 U/L. The authors concluded that "our study showed that BMD correlates negatively with BTMs and TRAP-5b presents a good specificity in identifying patients with postmenopausal osteoporosis" (Mederle et al., 2018).

Tian et al. (2019) performed a meta-analysis "to explore whether bone turnover biomarkers (BTMs), i.e., C-terminal telopeptide of type I collagen (CTX) and procollagen type I aminoterminal propeptide (PINP), are associated with fracture." Nine studies were included. PINP had a "significant" positive association with fracture (adjusted gradient risk [GR] = 1.28) after adjusting for confounders. CTX was also seen to associate with fracture (GR = 1.20). The authors concluded, "Our results indicate a statistically significant but modest association between BTMs (s-PINP or s-CTX) and future fracture risk after adjusting for BMD and clinical risk factors. The causal relationship between the two clinical conditions requires future validation with more standardized studies" (Tian et al., 2019).

Naylor et al. (2019) evaluated bone turnover markers (BTMs) ability to monitor "offset of treatment with bisphosphonates (BP) in osteoporosis." This was done by comparing the changes in BTMs and total hip (TH) bone mineral density (BMD). CTX and PINP were the BTMs analyzed, and offset was defined by "an increase greater than the least significant change (LSC) and an increase above the reference mean value." Fifty individuals were included, and at 48 weeks after stopping BPs, "CTX was greater than the LSC for 66% of the participants and PINP 72%; CTX was above the reference mean for 64% of the participants and PINP 42%." The authors also found that the decrease in TH-BMD was greater for those with the largest increases in BTMs, compared to those with "continued suppression." The authors concluded that "The measurement of BTM after withdrawal of BPs is potentially useful to evaluate patients that are taking a pause from treatment. An increase in BTMs more than the LSC and/or reference mean reflects loss of treatment effect and identifies patients that are likely to have a decrease in BMD" (Naylor et al., 2019).

Massera et al. (2019) evaluated the associations of osteocalcin (OC) and C-telopeptide of type I collagen (CTX) with "long-term incidence of hip fracture" in post-menopausal individuals. A total of 1680 individuals from the population-based Cardiovascular Health Study were included, and over a median follow-up period of 12.3 years, 288 hip fractures occurred. The authors found that increasing levels of CTX up to the middle-upper range (hazard ratio = 1.52 per standard deviation increase), with increases past this range only incrementally increasing risk (hazard ratio = 0.8). The authors identified an "inverted U-shaped relationship with incident fracture after adjustment" when comparing quartiles to each other, and an association was only seen for the quartile three to quartile one comparison (hazard ratio = 1.63). In a subset with "available measures," both OC and CTX were "inversely associated with bone mineral density of the hip." The authors concluded that "CTX, but not OC, levels were associated with incident hip fracture in post-menopausal individuals, a relationship characterized by an inverted U-shape" (Massera et al., 2019).

Migliorini et al. (2021) performed a systematic review of clinical trials reporting data on biomarkers for postmenopausal osteoporosis. A total of 36,706 patients were included from randomized trials. Data on biomarkers and clinical outcomes such as BMD, t-score, rate of fractures and adverse events were analyzed. Authors found that greater values of bone alkaline phosphatase (bALP) were associated with more vertebral and non-vertebral fractures. Greater values of urinary cross-linked N-telopeptides of type I collagen (NTx) at baseline were linked with an increase in adverse events at the last follow-up, and greater values of C-telopeptide of type I collagen at baseline were associated with more adverse events leading to discontinuation, gastrointestinal adverse events, musculoskeletal adverse events, and mortality. The authors concluded that the review "supports the adoption of BMTs during pharmacological therapy setting of patients suffering from osteoporosis" (Migliorini et al., 2021).

Wei et al. (2021) explored the relationship of procollagen type one N-terminal peptide (P1NP) and β cross-linked C-telopeptide of type one collagen (β-CTX) with bone mineral density (BMD) in postmenopausal individuals. All postmenopausal subjects "were selected from a community-based case-control study and P1NP and β-CTX were also collected and tested. The main correlation analysis was applied to explore the relationships of BMD, P1NP, and β-CTX." The results indicated that of the 1055 post-menopausal participants that were enrolled, "the BMD at all sites kept a decrease continually with age ($P < 0.01$). In addition, the level of β-CTX increased significantly from 45 to 50 years old and remained at a high level in the later stage, while the level of P1NP changed little or even decreased with age. Logistic regression model showed that β-CTX has better ability to predict BMD than P1NP, as demonstrated by an area under the curve (AUC) of 0.63." In conclusion, P1NP and β-CTX are important markers to monitor bone metabolism (Wei et al., 2021).

Guidelines and Recommendations

The Bone Health and Osteoporosis Foundation

In 2022, The Bone Health and Osteoporosis Foundation updated their guideline for the prevention and treatment of osteoporosis. Regarding biochemical markers of bone turnover, the guideline states:

Biochemical markers of bone turnover may:

- Predict rapidity of bone loss in untreated postmenopausal individuals.
- "Predict extent of fracture risk reduction when repeated after 3—6 months of treatment with FDA-approved therapies.
- Predict magnitude of BMD increases with FDA-approved therapies.
- Characterize patient compliance and persistence with osteoporosis therapy using a serum CTX for an antiresorptive medication and P1NP for an anabolic therapy (least significant change [LSC] is approximately a 40% reduction in CTX).
- Potentially be used during a bisphosphonate holiday to suggest when medication should be restarted, although more data are needed to support this recommendation"(LeBoff et al., 2022).

The North American Menopause Society (NAMS)

In 2021, the North American Menopause Society (NAMS) issued an updated position on the management of osteoporosis in postmenopausal individuals. NAMS stated:

"Bone turnover markers cannot diagnose osteoporosis and have varying ability to predict fracture risk in clinical trials. Bone turnover markers have been used primarily in clinical trials to demonstrate group responses to treatment" (NAMS, 2021).

"Although used by some osteoporosis specialists, the routine use of bone turnover markers in the evaluation of patients with osteoporosis is not recommended" (NAMS, 2021).

"Although changes in bone turnover markers are used by some specialists to assess adherence and effectiveness of therapy, routine use of bone markers is not recommended" (NAMS, 2021).

International Osteoporosis Foundation (IOF) and the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC)

In 2021, the IOF/IFCC published "*Practical Considerations for the Clinical Application of Bone Turnover Markers in Osteoporosis*"(Vasikaran et al., 2023). The authors concluded, "Serum PINP and β-CTX are useful for monitoring oral therapy in osteoporosis. Further studies for their application in managing offset of drug action after cessation of antiresorptive therapies with bisphosphonates and denosumab would be useful. Large-scale fracture risk prediction studies of PINP and β-CTX in various untreated population groups to assess how they interact with established risk factors used in risk calculators such as FRAX may help to include BTMs in such algorithms.

The B-ALP and TRACP-5b are least affected by renal failure and may be of potential use in assessment for osteoporosis in patients with CKD and monitoring such patients when treated. Studies of utility of TRACP-5b and B-ALP in fracture risk assessment as well as monitoring therapy and assessing offset of treatment effect in osteoporosis patients with CKD stages 3a-5D is warranted.

From an analytical point of view, standardization or harmonization of commercial assays for BTMs is important for collation of data from different studies and uniform application of decision limits and treatment targets in clinical guidelines. IOF-IFCC C-BM is pursuing these activities" (Vasikaran et al., 2023).

American Association of Clinical Endocrinologists and American College of Endocrinology

An update to the 2016 Guidelines for the Diagnosis and Treatment of Postmenopausal Osteoporosis was published in 2020. In it, the AACE/ACE state "Consider using bone turnover markers in the initial evaluation and follow-up of osteoporosis patients. Elevated levels can predict more rapid rates of bone loss and higher fracture risk", which is identical to the 2016 statement, but the 2020 edition is graded at an "A", up from "B" in 2016.

Similarly, the statement "Consider using bone turnover markers (BTMs) for assessment of patient compliance and efficacy of therapy. Significant reductions in BTMs are seen with antiresorptive therapy and have been associated with fracture reduction, and significant increases indicate good response to anabolic therapy" remains unchanged from the 2016 version, which was given a grade B.

Other relevant recommendations include:

- "Consider bone turnover markers at or below the median value for premenopausal [individuals] as a target for response to therapy for patients taking antiresorptive agents. Consider significant increases in bone formation markers as a pharmacologic response to anabolic therapy."

- "The ending of a bisphosphonate holiday should be based on individual patient circumstances such as… an increase in bone turnover markers."

Overall, although the joint guidelines acknowledge that BTMs cannot diagnose osteoporosis, they note that "elevated levels can predict more rapid rates of bone loss" and "are associated with increased fracture risk independent of BMD [bone mineral density] in some studies". Further, automated immunoassays have improved BTMs' reproducibility, and "changes in markers have been associated with bone response to therapy and reduction of fracture risk". Despite the numerous analytical issues with BTM assessment (lack of standardization, high cost, et al.), the guidelines note that some experts routinely use BTMs in clinical practice. They also note that the preferred bone turnover markers are PINP for bone formation and CTX for bone resorption. And, in the situations when patients might experience renal insufficiency or when there are insurance issues, then bone-specific alkaline phosphatase may be used. The guidelines conclude that "BTMs are useful in certain situations, such as assessment of fracture risk and to provide early feedback to patients that their drug is or is not working, which leads to discussions pertaining to medication compliance, drug absorption, and/or therapeutic efficacy. BTMs do not need to be assessed in all osteoporosis patients" (Camacho et al., 2020).

Consensus Group Report, managed by Scientific Advisory Board of European Society on Clinical and Economic Aspects of Osteoporosis, Osteoarthritis and Musculoskeletal Diseases

This working group was intended to "to provide guidance to clinicians on how to use BTMs in patient evaluation in postmenopausal osteoporosis, in fracture risk prediction and in the monitoring of treatment efficacy and adherence to osteoporosis medication." Their conclusions are listed below (Lorentzon et al., 2019):

- "The bone formation marker serum PINP [N-terminal collagen type I extension propeptide] and resorption marker serum βCTX-I [bone alkaline phosphatase for bone formation and C-terminal cross-linking telopeptide of type I collagen] are the preferred markers for evaluating bone turnover in the clinical setting."
- "Bone turnover markers cannot be used to diagnose osteoporosis but can be of value in patient evaluation and can improve the ability to detect some causes of secondary osteoporosis."
- "Serum βCTX-I and PINP correlate only moderately with bone loss in postmenopausal [individuals] and with osteoporosis medication-induced gains in BMD. Therefore, the use of bone turnover markers cannot be recommended to monitor osteoporosis treatment effect in individual patients."
- "Adding data on serum βCTX-I and PINP levels in postmenopausal [individuals] can only improve fracture risk prediction slightly in addition to clinical risk factors and BMD and therefore has limited value."
- "Bisphosphonates are the most commonly used osteoporosis medications, but adherence to oral bisphosphonates falls below 50% within the first year of treatment. Monitoring PINP and βCTX-I is effective in monitoring treatment adherence and can be defined as the sufficient suppression of these markers (by more than the LSC or to the lower half of the reference interval for young and healthy premenopausal [individuals])."

The guideline remarks "It is possible that monitoring the bone marker response may aid in the use of bisphosphonate treatment frequency and dosing when denosumab treatment is stopped."

The guideline also notes that a "systematic review of the present evidence concluded that there is insufficient evidence to recommend the use of monitoring bone turnover markers for predicting the effect of teriparatide treatment effect" (Lorentzon et al., 2019).

U.S. Preventative Services Task Force (USPSTF)

The 2018 USPSTF recommendation on screening to prevent osteoporotic fractures address clinical risk assessment and bone density measurement but do not mention bone turnover markers (Viswanathan et al., 2018).

Endocrine Society

The Endocrine Society released a guideline concerning pharmacological management of osteoporosis in postmenopausal individuals, which noted, "Monitoring bone turnover markers (serum C-terminal crosslinking telopeptide for antiresorptive therapy or procollagen type 1 N-terminal propeptide for bone anabolic therapy) is an alternative way of identifying poor response or nonadherence to therapy" (Eastell et al., 2019).

The Endocrine Society published an update to the above guideline in 2020, and the above statement concerning monitoring of bone turnover markers remained in the 2020 edition (Shoback et al., 2020).

The Endocrine Society also released guidelines regarding the management of Paget's Disease. They recommended "that in patients with increased bone turnover, biochemical follow-up should be used as a more objective indicator of relapse than symptoms" (Singer et al., 2014).

"For most patients, measurement of total alkaline phosphatase or other baseline disease activity markers at six to 12 weeks, when bone turnover will have shown a substantial decline, is an acceptable and cost-effective option" (Singer et al., 2014).

National Osteoporosis Guideline Group

The NOGG notes bone turnover markers (e.g., CTX, P1NP) as a possible measure to evaluate during investigation of osteoporosis/ fragility fractures (NOGG, 2021).

Kidney Disease Improving Global Outcomes (KDIGO): Mineral and bone disorder

The KDIGO released guidelines pertaining to bone turnover related to CKD.

- "In patients with CKD [stages] G3a–G5D, we suggest that measurements of serum PTH or bone-specific alkaline phosphatase can be used to evaluate bone disease because markedly high or low values predict underlying bone turnover."
- "In patients with CKD [stages] G3a–G5D, we suggest not to routinely measure bone-derived turnover markers of collagen synthesis (such as procollagen type I C-terminal propeptide) and breakdown (such as type I collagen cross-linked telopeptide, cross-laps, pyridinoline, or deoxypyridinoline)" (KDIGO, 2017).

The **Renal Association** also published a "commentary" on the KDIGO guidelines in 2018. In it, they remarked that "Although iPTH, whole PTH, and bALP levels were associated with bone turnover, no biomarker singly or in combination was sufficiently robust to diagnose low, normal, and high bone turnover in an individual patient [on dialysis]" (Burton et al., 2018).

Fourth International Workshop on the Management of Asymptomatic Primary Hyperparathyroidism

This workshop published guidelines regarding management of asymptomatic primary hyperparathyroidism (PHPT). They note bone turnover markers as an optional measurement of asymptomatic PHPT, listing "bone-specific alkaline phosphatase activity, osteocalcin, P1NP [select one]; serum CTX, urinary NTX [select one]" (Bilezikian et al., 2014).

International Osteoporosis Foundation and European Society for Clinical and Economic Aspects of Osteoporosis and Osteoarthritis

The IOF/ESCEO issued joint guidelines stating the following (Kanis et al., 2018):

"Bone markers (serum procollagen type I N propeptide (s-PINP) and serum C-terminal cross-linking telopeptide of type I collagen (s-CTX) as markers of bone formation and bone resorption, respectively) have some prognostic significance for fracture in situations where bone mineral density (BMD) is unavailable."

The joint guidelines also note that if harmonization efforts for other bone turnover markers are successful, these markers may see use for fracture risk. Procollagen I N-terminal peptide (P1NP) and C-telopeptide breakdown products (especially serum CTX) are considered the most informative biochemical markers for monitoring of osteoporosis (Kanis et al., 2018).

International Osteoporosis Foundation (IOF), Asian Federation of Osteoporosis Societies (AFOS), and the International Society for Clinical Densitometry (ISCD)

The IOF Capture the Fracture program facilitates the establishment of Fracture Liaison Services (FLS) with a goal "properly identify and treat patients with fragility fractures, improve quality of post-fracture care, adherence, and prevention of secondary fractures worldwide, including the [Asia-Pacific] region." In 2021, the IOF, AFOS, and ISCD endorsed a consensus statement on the use of BTMs in the Asia-Pacific region. They made the following consensus statements (Wu et al., 2021):

- "Endorse the use of BTMs, especially CTX and P1NP, as short-term monitoring tools for osteoporosis treatment, consistent with recommendations of the AACE/ACE, IOF, IFCC, JOS, NOF, TOA, and associated organizations.
- BTMs can be used to differentiate patients with relatively higher or lower bone turnover rates and thereafter, helping clinicians to choose an appropriate anti-osteoporosis treatment regimen.
- BTMs can reflect the therapeutic responses to anti-osteoporosis therapies earlier than BMD and are therefore of help both in selecting osteoporosis treatment and in assessing its responses to therapies.
- Absolute values or the degree of change from baseline for BTMs can be used to monitor the efficacy of osteoporosis therapies clinically.
- CTX and/or P1NP can be used to evaluate patient adherence and drug responses to anti-resorptive agents, with measurements suggested at baseline, three months, six months, and 12 months after starting treatment.
- P1NP can be used to evaluate patient adherence and drug responses to anabolic agents, with measurements at baseline, one to three months, six months, and 12 months after starting anabolic treatment.
- Encourage reimbursement of BTMs by different health insurance programs in the Asia-Pacific to improve patient adherence and treatment outcomes.

- Recommend appropriate use of BTMs as a short-term monitoring tool for improving the use of therapeutic regimens in osteoporosis care programs, such as fracture liaison service (FLS)."

They conclude that "the use of BTMs can be incorporated in treatment algorithms of osteoporosis care programs to improve patient adherence and treatment outcomes" (Wu et al., 2021).

The International Federation of Clinical Chemistry and Laboratory Medicine

In the most recent review of bone turnover markers for the journal of the International Federation of Clinical Chemistry and Laboratory Medicine the author Bhattoa (2018) found that "Although quite sensitive to a multitude of exogenous and endogenous pre-analytical factors, bone markers are best used in monitoring anti-osteoporosis therapy efficacy and compliance. Combination of BMD measurement by DEXA with biochemical markers of bone turnover levels, at least one bone resorption and one bone formation marker, may potentially improve early detection of individuals at increased risk for bone loss and eventually non-traumatic bone fracture. Furthermore, they have widespread clinical utility in osteoporosis, renal osteodystrophy, certain oncological conditions and rheumatic diseases" (Bhattoa, 2018).

International Society for Clinical Densitometry (ISCD)

The ISCD includes a comment on bone turnover markers in their guideline titled "Official Positions", stating that "Serial BMD [bone mineral density] testing in combination with clinical assessment of fracture risk, bone turnover markers and other factors…can be used to determine whether treatment should be initiated in untreated patients, according to locally applicable guidelines" (ISCD, 2019).

Royal Australian College of General Practitioners (RACGP)

In 2013, the RACGP released a series of "Tests and results" aimed at providing information about common tests that general practitioners order regularly. The series focused on areas such as indications, what to tell the patient, what the test can and cannot tell you, and interpretation of the results. As an assessment of fracture risk, they note that "Bone turnover markers increase in proportion to fracture risk, independent of bone mineral density (BMD). In general, turnover markers also tend to be higher in patients with low bone density. However, this correlation is not absolute in individuals and this application of the test is most useful in population studies. Very high marker levels (more than 1.5 times the upper reference limit) are not typical of postmenopausal osteoporosis and should prompt a search for another cause. For example, after a fracture, markers may remain increased for up to six months. Other causes could include high turnover states such as hyperparathyroidism or hyperthyroidism, Paget disease, malignancy including myeloma, or advanced renal failure" (Coates, 2013).

As a method of monitoring the efficacy of osteoporosis treatment, BMD "is a common surrogate marker of osteoporosis treatment efficacy. However, due to the relatively small effect of treatment relative to the precision of the test, it is not practical to repeat BMD at intervals shorter than two years. Also, fracture risk reduction on treatment is far greater than would be predicted by the BMD increase achieved. Fewer than half of patients prescribed a bisphosphonate are taking the medication after 1 year. For these reasons, it is helpful to assess the effects of, and compliance with, treatment within a few months. Some studies show improved adherence to treatment when turnover marker results were provided to patients, although this finding is not universal" (Coates, 2013).

Overall, the RACGP's guideline for osteoporosis management recognizes "the response of bone turnover markers to treatment, particularly in the first few months after initiating bisphosphonates or teriparatide, but does not yet recommend their routine use" (Coates, 2013).

Paget's Association, Guideline Development Group

This Guideline Development Group published a guideline titled "Diagnosis and Management of Paget's Disease of Bone in Adults". The relevant remarks include (Ralston et al., 2019):

- "Serum total ALP [total alkaline phosphatase] is widely available and considerably cheaper than other biochemical markers that have been assessed in PDB [Paget's Disease of Bone]."
- "If total ALP values are normal and clinical suspicion of metabolically active PDB is high, measurement of BALP, PINP, or uNTX may be considered to screen for metabolically active disease."
- "…elevations in markers of bone turnover occur in many disease states and cannot be used in isolation for the diagnosis of PDB."
- "Measurement of PINP is recommended to predict lesion extent, as defined by scintigraphy, after bisphosphonate therapy."
- "Measurement of biochemical markers of bone turnover are not recommended a means of predicting the response of bone pain to osteoclast inhibitors in PDB" (Ralston et al., 2019).

Royal Osteoporosis Society (ROS)

In 2021, the ROS updated a 2018 a statement on the use of bone markers and osteoporosis. In it, they included three reasons as to why bone markers may be used (Royal Osteoporosis Society, 2021):

> "a) To measure bone turnover as part of an assessment of bone strength and fracture risk. There haven't been many research trials to prove how effective this is, so other methods are usually used to assess bone strength, including a bone density scan to measure your bone density, along with your other risk factors.
> b) To monitor the effectiveness of osteoporosis drug treatments. Most treatments work by slowing the rate of bone resorption. The rate of bone formation also slows, but the overall effect is that the two processes come back into balance, leading to improved bone strength. The effect of a drug treatment on bone turnover can be assessed using bone markers within six months of starting treatment.
> c) In research trials, to assess osteoporosis drugs in development. Although there is evidence to suggest the value of bone marker tests as outlined above, expert opinion is divided on how useful or necessary they are, and further research is required to establish how they should be best used in the management of osteoporosis."

As to the recommendation on the use of bone markers, they noted that a "UK independent review in 2014, which looked at whether bone markers should be used to see if a drug treatment is working, concluded there was insufficient evidence available to make recommendations. International expert guidance, however, says that although more research is needed, bone markers can be useful in some situations" (Royal Osteoporosis Society, 2021).

Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations

(NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Several tests for bone turnover markers have been cleared by the U.S. Food and Drug Administration (FDA) using the 510(k) process including the collagen cross-links tests: Osteomark® NTX Urine ELISA test from Abbott which measures cross-linked N-telopeptides of type 1 collagen (NTx), and Serum Crosslaps One-step ELISA test which measures hydroxyproline. Other bone turnover marker tests cleared through the FDA 510(k) process tests include: Access Ostase from Beckman Coulter which measures bone-specific alkaline phosphatase (B-ALP), N-MID Osteocalcin One-step ELISA from Osteometer Bio Tech (merged with Osteopro and now called Nordic Biotech) which measures osteocalcin (OC), and Elecsys® N-MID Osteocalcin Immunoassay (Roche Diagnostics).

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
82523	Collagen cross links, any method
83500	Hydroxyproline; free
83505	Hydroxyproline; total
83937	Osteocalcin (bone gla protein)
84080	Phosphatase, alkaline; isoenzymes

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

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Revision History

Review Date	Summary of Changes
09/06/2023	Reviewed and Updated: Updated the background, guidelines and recommendations, and evidence-based scientific references. Literature review necessitated the following changes to coverage criteria: Removal of CC1: "1) For osteoporosis patients, measurement of bone turnover markers MEETS COVERAGE CRITERIA for the following situations: a) In initial evaluation and management b) In fracture risk prediction" BTM is recommended for fracture risk prediction in individuals who are receiving treatment for their osteoporosis. Adjustment of former CC2, now CC1, to address frequency of testing in individuals receiving bisphosphonate treatment. "1) For individuals treated with bisphosphonates,

	measurement of bone turnover markers to assess an individual's compliance with bisphosphonate therapy MEETS COVERAGE CRITERIA at the following intervals: a) To establish baseline levels before initiating bisphosphonate treatment b) Every three months after initiation or change of therapy for the first year. c) Every two years when no medication changes have occurred." All other CC except no CC3 edited for clarity and consistency.

Cardiovascular Disease Risk Assessment

Policy Number: AHS – G2050 – Cardiovascular Disease Risk Assessment	Prior Policy Name and Number, as applicable: 1. AHS – G2010 – Lipid Panels 2. AHS – G2050 – Novel Biomarkers in Risk Assessment and Management of Cardiovascular Disease 3. AHS – G2053 – Cardiovascular Risk Panels 4. AHS – G2106 – Measurement of Serum Intermediate Density Lipoproteins 5. AHS – M2082 – Measurement of Lipoprotein, Associated Phospholipase A2 6. AHS – G2104 – Measurement of Long-Chain Omega-3 Fatty Acids 7. AHS – G2096 – Homocysteine Testing 8. AHS – M2090 – Genotyping for 9P21 Single Nucleotide Polymorphisms 9. AHS – M2102 – KIF6 Genotyping for Predicting Cardiovascular Risk and/or Effectiveness of Statin Therapy 10. AHS – M2064 – Genetic Expression to Predict Coronary Artery Disease
Initial Presentation Date: 09/19/2016 Effective Date: 02/01/2025	

Policy Description

Cardiovascular risk assessment comprises the means and processes to predict the probability of developing a cardiovascular disease. These are a group of tests and health factors that have been proven to indicate a person's chance of having a cardiovascular event such as a heart attack or stroke.

Tests typically used to assess cardiovascular risk include lipid profiles or panels, biomarkers, and cardiovascular risk panels.

For guidance concerning lipid screening in individuals under 18 years of age, please refer to AHS-G2042-Pediatric Preventive Screening.

For guidance concerning homocysteine testing for indications other than cardiovascular disease, please refer to AHS-M2141-Testing of Homocysteine Metabolism-Related Conditions and AHS-G2014-Vitamin B12 and Methylmalonic Acid Testing.

Terms such as male and female are used when necessary to refer to sex assigned at birth.

Related Policies

Policy Number	Policy Title
AHS-G2014	Vitamin B12 and Methylmalonic Acid Testing
AHS-M2141	Testing of Homocysteine Metabolism-Related Conditions
AHS-M2180	Genetic Markers for Cardiovascular Disease Risk Assessment

Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) Measurement of apolipoprotein B (apoB) **MEETS COVERAGE CRITERIA** for **any** of the following situations:
   a) For individuals with hypertriglyceridemia.
   b) For individuals with diabetes mellitus.
   c) For individuals with obesity or metabolic syndrome.
   d) For individuals with other dyslipidemias (such as very low LDL-C).
   e) For individuals who are on lipid therapy.
   f) For individuals who are suspected to have familial dysbetalipoproteinemia or familial combined hyperlipidemia.

2) For individuals 18 years of age or older, measurement of lipoprotein a (Lp(a)) once per lifetime **MEETS COVERAGE CRITERIA**.

3) For individuals for whom a risk-based treatment decision is uncertain (after quantitative risk assessment using ACC/AHA PCEs to calculate 10-year risk of CVD events [see Note 2]), testing for C-reactive protein with the high-sensitivity method (hs-CRP) **MEETS COVERAGE CRITERIA** at the following frequency:
   a) For initial screening, two measurements at least two weeks apart.
   b) If the initial screen was abnormal, follow-up screening is allowed up to once per year.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

4) For all other cardiovascular disease risk assessments not described above, testing for CRP **DOES NOT MEET COVERAGE CRITERIA**.

5) For CVD risk assessment and stratification in the outpatient setting, measurement of high-sensitivity cardiac troponin T (hs-cTnT) **DOES NOT MEET COVERAGE CRITERIA**.

6) For CVD risk assessment screening, evaluation, and management, homocysteine testing **DOES NOT MEET COVERAGE CRITERIA**.

7) For CVD risk assessment, measurement of novel lipid and non–lipid biomarkers (e.g., apolipoprotein AI, apolipoprotein E, B-type natriuretic peptide, cystatin C, fibrinogen, leptin, LDL subclass, HDL subclass) **DOES NOT MEET COVERAGE CRITERIA**.

8) Other than simple lipid panels (see Note 1), CVD risk panels consisting of multiple individual biomarkers intended to assess CVD **DO NOT MEET COVERAGE CRITERIA**.

9) For CVD risk assessment, measurement of serum intermediate density lipoproteins **DOES NOT MEET COVERAGE CRITERIA**.

10) For CVD risk assessment, measurement of lipoprotein-associated phospholipase A2 (Lp-PLA2) **DOES NOT MEET COVERAGE CRITERIA**.

11) For all situations, measurement of long-chain omega-3 fatty acids in red blood cell membranes, **DOES NOT MEET COVERAGE CRITERIA**.

12) All other tests for assessing CVD risk **DO NOT MEET COVERAGE CRITERIA**.

NOTES:

Note 1: A simple lipid panel is generally composed of the following lipid markers:

- Total cholesterol
- HDL cholesterol
- LDL cholesterol
- Triglycerides

Certain calculated ratios, such as the total/HDL cholesterol may also be reported as part of a simple lipid panel.

Other types of lipid testing (i.e., apolipoproteins, lipid particle number or particle size, lipoprotein [a]) are not considered to be components of a simple lipid profile.

Note 2: 2013 ACC/AHA Guideline on the Assessment of Cardiovascular Risk (Goff et al., 2014):
Risk factors include gender, age, race, smoking, hypertension, diabetes, total cholesterol, high- and low-density lipoprotein cholesterol. A race- and sex-specific PCE ASCVD Risk Estimator is available at:

https://tools.acc.org/ldl/ascvd_risk_estimator/index.html#!/calulate/estimator/.

The 2018 AHA/ACC/AACVPR/AAPA/ABC/ACPM/ADA/AGS/APhA/ASPC/NLA/PCNA Guideline on the Management of Blood Cholesterol affirms that "the PCE is a powerful tool to predict population risk, but it has limitations when applied to individuals." Hence a clinician-patient risk discussion can individualize risk status based on PCE, but with the inclusion of additional risk-enhancing factors. These additional factors may include:

- A family history of premature ASCVD (males, age <55 y; females, age <65 y)
- Primary hypercholesterolemia (LDL-C, 160–189 mg/dL [4.1–4.8 mmol/L); non–HDL-C 190–219 mg/dL [4.9–5.6 mmol/L])
- Metabolic syndrome (increased waist circumference, elevated triglycerides [>150 mg/dL], elevated blood pressure, elevated glucose, and low HDL-C [<40 mg/dL in men; <50 in women mg/dL] are factors; tally of 3 makes the diagnosis)
- Chronic kidney disease (eGFR 15–59 mL/min/1.73 m2 with or without albuminuria; not treated with dialysis or kidney transplantation)
- Chronic inflammatory conditions such as psoriasis, RA, or HIV/AIDS
- History of premature menopause (before age 40 y) and history of pregnancy-associated conditions that increase later ASCVD risk such as preeclampsia
- High-risk race/ethnicities (eg, South Asian ancestry)
- Lipid/biomarkers: Associated with increased ASCVD risk
- Persistently elevated, primary hypertriglyceridemia (≥175 mg/dL)
- Elevated high-sensitivity C-reactive protein (≥2.0 mg/L)
- Elevated Lp(a): A relative indication for its measurement is family history of premature ASCVD. An Lp(a) ≥50 mg/dL or ≥125 nmol/L constitutes a risk-enhancing factor especially at higher levels of Lp(a)
- Elevated apoB ≥130 mg/dL: A relative indication for its measurement would be triglyceride ≥200 mg/dL. A level ≥130 mg/dL corresponds to an LDL-C ≥160 mg/dL and constitutes a risk-enhancing factor
- ABI <0.9

Table of Terminology

Term	Definition
AA	Arachidonic acid
AACE	American Association of Clinical Endocrinologists
AACVPR	American Association of Cardiovascular and Pulmonary Rehabilitation
AAPA	American Academy of Physician Assistants
ABC	Association of Black Cardiologists
ABI	Ankle-brachial index
ACC	American College of Cardiology
ACCF	American College of Cardiology Foundation
ACE	American College of Endocrinology
ACPM	American College of Preventive Medicine
ADA	American Diabetes Association
ADMA	Asymmetric dimethylarginine
AGS	American Geriatrics Society

AHA	American Heart Association
Anti-CCP	Anti–cyclic citrullinated peptide
APhA	American Pharmacists Association
Apo A-I	Apolipoprotein A-I
Apo B	Apolipoprotein B
Apo C-III	Apolipoprotein C-III
Apo E	Apolipoprotein E
APs	Antipsychotics
ART	Antiretroviral therapy
ASCP	American Society for Clinical Pathology
ASCVD	Atherosclerotic cardiovascular disease
ASH	American Society of Hypertension
ASPC	American Society for Preventive Cardiology
BMI	Body mass index
BNP	Brain natriuretic peptide
CAC	Coronary artery calcium
CAD	Coronary artery disease
CCS	Canadian Cardiovascular Society
CDC	Centers for Disease Control and Prevention
CHARGE	Cohorts for Heart and Aging Research in Genomic Epidemiology
CHD	Coronary heart disease
CIMT	Carotid intima media thickness
CK	Creatine kinase
CKD	Chronic kidney disease
CRP	C reactive protein
cTnI	Cardiac troponin I
cTnT	Cardiac troponin T
CVD	Cardiovascular disease
DHA	Docosahexaenoic acid
DM	Diabetes mellitus
DoD	Department of Defense
DPA	Docosapentaenoic acid
EACPR	European Association for Cardiovascular Prevention & Rehabilitation
EAS	European Atherosclerosis Society
EASD	European Association for the Study of Diabetes
EEG	Electroencephalography
ELISA	Enzyme-linked immunosorbent assay
EPA	Eicosapentaenoic acid
ES	Endocrine Society
ESC	European Society of Cardiology
FDA	Food and Drug Administration

FH	Familial hypercholesterolemia
Fix	Family history
GDMT	Guideline-directed medical therapy
GFR	Glomerular filtration rate
GGT	Gamma-glutamyltransferase
GlycA	Glycoprotein acetyls
HDL	High-density lipoprotein
HDL-C	High-density lipoprotein cholesterol
HF	Heart failure
HIV	Human immunodeficiency virus
HRs	Hazard ratios
hsCRP	High-sensitivity C reactive protein
hs-cTnI	High-sensitivity cardiac troponin i
hs-cTnT	High-sensitivity cardiac troponin t
IDL	Intermediate density lipoproteins
LDL	Low-density lipoprotein
LDL-C	Low-density lipoprotein cholesterol
LDTs	Laboratory developed tests
Lp(a)	Lipoprotein A
LPA	Apolipoprotein(a) locus
Lp-PLA2	Lipoprotein-associated Phospholipase A2
LSP	Lipids Standardization Program
MACE	Major adverse cardiovascular event
NICE	National Institute for Health and Care Excellence
NLA	National Lipid Association
NMA	National Medical Association
Non-HDL-C	Non-high-density lipoprotein cholesterol
NT-proBNP	Amino-terminal pro-B-type natriuretic peptide
PAD	Peripheral artery disease
PCEs	Pooled cohort equations
PCNA	Preventive Cardiovascular Nurses Association
proBNP	Pro-B-type natriuretic peptide
RR	Risk ratio
sdLDL	Small dense low-density lipoprotein
SDMA	Symmetric dimethylarginine
SIGN	Scottish Intercollegiate Guidelines Network
SMC™	Single Molecule Counting
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus
TC	Total cholesterol
TG	Triglyceride

USPSTF	U.S. Preventive Services Task Force
VA	Veterans Affairs
VLDL	Very low-density lipoprotein

Scientific Background

Statistics show that cardiovascular disease (including coronary heart disease, stroke, and hypertension) is America's leading health problem, and the leading cause of death. According to the 2024 update of the heart disease and stroke statistics report released by the American Heart Association (AHA, 2024):

- Approximately 127.9 million people in this country suffer from some form of cardiovascular disease (encompassing coronary heart disease, heart failure, hypertension, and stroke).
- The direct and indirect costs of total cardiovascular disease are about $422.3 billion and increasing every year.
- An estimated 122.4 million U.S. adults have hypertension, and 29.3 million have diabetes.
- Heart disease remains the number one cause of death in the U.S.
- On average, someone in the U.S. dies of a stroke every 3 minutes and 14 seconds.
- Women have a higher lifetime risk of stroke than men.
- Approximately 14 percent of U.S. adults smoke cigarettes “some days” or “every day”.
- An estimated 42.4 percent of U.S. adults are obese

Cardiovascular Risk Assessment

Traditionally, the most important indicators for cardiac risk are those of a person's health history. These include factors such as family history, age, weight, exercise, and cigarette smoking status (Wilson, 2024b).

Tests typically used to assess cardiovascular risk include:

1. Lipid profile or panel, which is the most important blood test for cardiac risk assessment
2. Biomarkers
3. Cardiovascular Risk Panels

Lipid Profile or Panel

A lipid profile or lipid panel is a panel of blood tests that serves as an initial broad medical screening tool for abnormalities in lipids, such as cholesterol and triglycerides. The results of this test can identify certain genetic diseases and can determine approximate risks for cardiovascular disease and other diseases. The lipid profile typically includes measurements of low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglycerides, and total cholesterol. Using these values, a laboratory may also calculate the very low-density lipoprotein (VLDL) and total cholesterol/HDL cholesterol ratio (Rosenson, 2022).

Biomarkers

Traditional risk algorithms may miss up to 20% of cardiovascular disease (CVD) events (MacNamara et al., 2015). Numerous biomarkers have been proposed as potential risk markers for CVD. These biomarkers include but are not limited to several apolipoproteins (A, B, AI, E, LDL, HDL), B-type natriuretic peptide, and C-reactive protein. These biomarkers have been proposed as an alternative or addition for risk stratification in CVD or as treatment targets for lipid-lowering therapy (R. Rosenson,

2023; Rosenson et al., 2024; Wilson, 2024a). However, even the most promising biomarkers have only demonstrated modest associations and predictive ability.

Antonopoulos et al. (2022) investigated the added prognostic value of biomarkers of vascular inflammation for stable patients without known coronary heart disease on top of clinical risk factors. The biomarkers—"C-reactive protein, interleukin-6 and tumor necrosis factor-a, arterial positron emission tomography/computed tomography and coronary computed tomography angiography–derived biomarkers of vascular inflammation, including anatomical high-risk plaque features and perivascular fat imaging"—were evaluated against the main endpoint of the difference in c-index (Δ[c-index]) with the use of inflammatory biomarkers for major adverse cardiovascular events (MACEs) and mortality, finding that the "Biomarkers of vascular inflammation provided added prognostic value for the composite endpoint and for MACEs only (pooled estimate for Δ[c-index]% 2.9, 95% CI: 1.7-4.1 and 3.1, 95% CI: 1.8-4.5, respectively)." Coronary computed tomography angiography-related biomarkers are noteworthy as they produced the highest added prognostic benefit for MACEs, the biomarkers of which included "high-risk plaques 5.8%, 95% CI: 0.6 to 11.0, and perivascular adipose tissue (on top of coronary atherosclerosis extent and high-risk plaques): 8.2%, 95% CI: 4.0 to 12.5)." Though this may appear to be the case, the study remarked that the net clinical benefit and cost-effectiveness of using these biomarkers are still underreported and understudied (Antonopoulos et al., 2022).

Since low-grade inflammation has been linked to early development of cardiovascular disease in the young, Chiesa et al. (2022) evaluated whether circulating levels of glycoprotein acetyls (GlycA) were better able to predict the development of adverse cardiovascular disease risk profiles when compared with the more commonly used biomarker high-sensitivity CRP (C-reactive protein). Using data from a total of 3306 adolescents and young adults from the Avon Longitudinal Study of Parents and Children (mean age, 15.4 ± 0.3; n=1750) and Cardiovascular Risk in Young Finns Study (mean age, 32.1 ± 5.0; n=1556), the authors found that not only did "GlycA showed greater within-subject correlation over 9-to-10-year follow-up [for hypertension and metabolic syndrome] in both cohorts compared with CRP, particularly in the younger adolescent group (r=0.36 versus 0.07)," but GlycA was associated with multiple lifestyle-related cardiovascular disease risk factors, cardiometabolic risk factor burden, and vascular dysfunction. Moreover, in both cohorts, "only GlycA predicted future risk of both hypertension (risk ratio [RR], ≈1.1 per z-score increase for both cohorts) and metabolic syndrome (RR, ≈1.2–1.3 per z-score increase for both cohorts) in 9-to-10-year follow-up," suggesting that GlycA "may capture distinct sources of inflammation in the young and may provide a more sensitive measure than CRP for detecting early cardiovascular risk" (Chiesa et al., 2022).

Apolipoprotein B (Apo B)

Apolipoprotein B is a major protein in the construction and regulation of lipids. There are two forms of apo B, apo B-48 and apo B-100. Apo B-100 is the major protein found in LDL and VLDL. Each LDL particle has one molecule of Apo B-100 per particle. Therefore, the apo B concentration may represent the amount of LDL well (R. Rosenson, 2023). Increased levels of apo B have been associated with atherosclerosis development in several large-scale studies; however, apo B levels have yet to become routinely measured in clinical practice (Morita, 2016; Trompet et al., 2018).

Researchers have hypothesized that lowering apo B levels in young or middle-aged individuals will reduce the number of atherosclerosis cases later in life (Robinson et al., 2018). Further, atherosclerotic changes in retinal arteries have been associated with coronary artery disease (CAD) as well as apo B, TG, TC, and LDL-C levels (Tedeschi-Reiner et al., 2005). Lamprea-Montealegre et al. (2020) have analyzed data from 9270 participants with chronic kidney disease to determine if triglyceride-rich lipoproteins

contribute to a greater CVD risk in this population; it was determined that increased apo B along with other triglyceride and cholesterol-related concentrations were associated with an increased risk for atherosclerotic CVD risk in chronic kidney disease patients. A second study (n=8570) has researched the relationship between apo B levels relative to LDL-C and non-LDL-C, as well as how these levels affect subclinical atherosclerotic cardiovascular disease (ASCVD) (Cao et al., 2019). Results showed that higher apo B levels were associated with an increase in coronary artery calcium (CAC) levels among adults older than 45 years who were not taking statins, "but provided only modest additional predictive value of apo B for CAC prevalence, incidence, or progression beyond LDL-C or non-HDL-C" (Cao et al., 2019). An equation to predict major cardiovascular events based on apo B levels has even been developed, and when studied, this equation showed major cardiovascular event "risk prediction comparable to directly-measured apo B in high risk patients with previous coronary heart disease" (Hwang et al., 2017).

In 2019, European Society of Cardiology (ESC) and European Atherosclerosis Society (EAS) published guidelines for the management of dyslipidaemias. These guidelines stated that "ApoB analysis is recommended for risk assessment, particularly in people with high TG [triglycerides], Diabetes mellitus (DM), obesity or metabolic syndrome, or very low LDL-C" (Mach et al., 2019). The ESC and EAS justify these recommendations by stating that the measurement of LDL-C levels in patients with dyslipidaemia may be inaccurate due to high DM or TG levels. "Because apo B provides an accurate estimate of the total concentration of atherogenic particles under all circumstances, it is the preferred measurement to further refine the estimate of ASCVD risk that is modifiable by lipid-lowering therapy" (Mach et al., 2019).

Apolipoprotein A-I (Apo-A-I)

Apolipoprotein A-I is a lipid-binding protein which comprises HDL molecules. HDL contains two associated apolipoproteins, A-I and A-II, and together they are the primary components of the HDL molecules. Due to Apo A-I's role as a primary structural protein for HDL, it significantly factors into the density ranges of HDL, which ultimately contribute to their overall measurement (R. Rosenson, 2023).

Direct measurement of apo A-I has been proposed as more accurate than the traditional use of HDL level. Low levels of apo A–I may be associated with an increased risk for CVD. Testing for apo A–I is often performed with apolipoprotein B and reported as a ratio (apo B: apo A-I), thus providing a measure of atherogenic to antiatherogenic lipoprotein particles (Sandhu et al., 2016).

Apolipoprotein E (Apo E)

Apolipoprotein E is the primary apolipoprotein found in VLDLs and chylomicrons. Apo E is essential in the metabolism of cholesterol and triglycerides and helps to clear chylomicrons and VLDL. Apo E polymorphisms have functional effects on lipoprotein metabolism. Some Apo E genotypes are more atherogenic than others, and their measurement could provide additional information of risk of coronary artery disease (R. Rosenson, 2023).

B-type or Brain Natriuretic Peptide (BNP)

Brain Natriuretic Peptide is a hormone released by the ventricles of the heart when pressure to the cardiac muscles increases or there is volume overload. BNP is now an established biochemical marker for heart failure, as the level of BNP in plasma increases proportionally based on disease severity (Kuwahara et al., 2018). Further, BNP has been accepted as an "independent surrogate marker of rehospitalization and death" for heart disease (Li & Wang, 2005), and exhibits both diagnostic and prognostic capabilities (Tomcsányi et al., 2018).

While BNP has shown great promise for diagnostic congestive heart failure purposes, a BNP guided heart failure treatment strategy seems to be controversial; some report that this type of treatment has led to greater health-related costs and does not increase patient outcomes (Mark et al., 2018). Still, many drugs, such as beta blockers, amiodarone, spironolactone, and angiotensin converting enzyme inhibitors, have been beneficial in reduction of circulating BNP during the management of chronic heart failure (Li & Wang, 2005). A major limitation of BNP is that a wide range of values are observed in patients with and without heart failure; for example, obese individuals tend to have lower levels of this hormone than healthy individuals (Colucci & Chen, 2024).

Januzzi et al. (2019) used data from the GUIDing Evidence Based Therapy Using Biomarker Intensified Treatment in Heart Failure (GUIDE-IT) trial to develop a greater understanding of the prognostic capabilities of amino-terminal pro-B-type natriuretic peptide (NT-proBNP) following heart failure. A total of 638 individuals participated in the study. The authors concluded that "Patients with heart failure with reduced ejection fraction whose NT-proBNP levels decreased to ≤1,000 pg/ml during GDMT [guideline-directed medical therapy] had better outcomes" (Januzzi et al., 2019). These results highlight the potential for NT-proBNP to be used as a prognostic tool following heart failure.

High Density Lipoprotein (HDL)

Apart from apolipoprotein content (AI and AII), HDL can be classified by size (small and large), by density (HDL2, HDL3), and by electrical charge (pre-beta, alpha and pre-alpha). There has been substantial interest in evaluating whether HDL subclass testing can be used to provide additional information on cardiovascular risk compared to HDL alone. HDL levels have been noted to be inversely related to CVD risk and possibly even protective against CVD. However, there are still many questions about the relationship between HDL and CVD risk, such as whether HDL levels are causative of lower CVD risk (R. Rosenson, 2023; Rosenson & Durrington, 2024).

Low Density Lipoprotein (LDL)

Low Density Lipoprotein proteins are a significant risk factor in predicting atherosclerosis. The mechanism of how LDL subclass particles impact risk of CVD has not been determined although many mechanisms have been proposed. Even though LDL cholesterol levels may be normal, an elevation of small, dense LDL particles may be associated with CVD. One theory is that the small LDL particles can be more easily deposited into the intima, lead to atherosclerosis, and eventually CVD. Another is that LDL particles may upregulate the angiotensin II receptor, thereby promoting atherosclerosis (R. Rosenson, 2023).

Lipoprotein(a)

Lipoprotein(a) (Lp[a]) is a low-density lipoprotein and has been determined to have atherogenic potential. Lp(a) has been proposed as an independent risk factor for coronary artery disease (CAD). Although research has shown it accumulates in atherosclerotic lesions, the actual process remains unclear. Serum levels of Lp(a) are highly determined by genetic polymorphisms, and the 90th percentile of Lp(a) levels was estimated at about 39 mg/dL. The overall degree of risk associated with Lp(a) levels appears to be modest, and the degree of risk may be mediated by other factors such as LDL levels and/or hormonal status. The standard method for measuring Lp(a) is density gradient ultracentrifugation. Although enzyme-linked immunosorbent assay (ELISA) techniques are available; they unable to distinguish between apo(a) isoforms, leading to inaccurate results (Rosenson et al., 2024). Lp(a) may have prognostic value in certain situations, such as in individuals with hypercholesterolemia

(Grundy et al., 2018).

A study focusing on the possible role of Lp(a) in CVD was performed by Willeit et al. (2018); 26069 subjects were analyzed, and the authors found a linear relationship between elevated Lp(a) levels and CVD risk at a baseline of ≥30 mg/dL and an on-statin level of ≥50 mg/dL. The baseline hazard ratios were 1.13 and 1.36 for 30-50 mg/dL and >50 mg/dL respectively, and the hazard ratios for patients on statins were 1.08 and 1.42 respectively (Willeit et al., 2018).

Mehta et al. (2020) investigated "independent and joint associations of Lp(a) and FHx [family history of coronary heart disease] with atherosclerotic cardiovascular disease (ASCVD) and CHD [coronary heart disease] among asymptomatic subjects." A total of 12149 patients were included and observed over 21 years, with Lp(a) levels measured at the first visit and remeasured at the fourth visit, nine years later, to confirm. The median age of this cohort was 54 years, and 44% of these patients had FHx. A total of 3114 ASCVD events were observed. Both FHx and elevated Lp(a) were independently associated with ASCVD, with a hazard ratio of 1.17 for FHx and a hazard ratio of 1.25 for elevated Lp(a). Patients with both FHx and elevated Lp(a) were found to have a hazard ratio of 1.43. Similar findings were found for CHD. The authors also noted that ASCVD and CHD risk reclassification and discrimination indices had improved accuracy with both FHx and Lp(a) included. The authors concluded that "elevated plasma Lp(a) and FHx have independent and additive joint associations with cardiovascular risk and may be useful concurrently for guiding primary prevention therapy decisions" (Mehta et al., 2020).

Cystatin C

Cystatin C is a protease inhibitor protein that plays a role in inflammation and obesity. Serum testing has been proposed to diagnose impaired kidney function, which in turn may be a risk factor for coronary heart disease (Rule, 2022). There is no published literature proving the effectiveness of Cystatin C as a biomarker for predicting cardiovascular risk and other confounding factors such as inflammation levels still need to be parsed out from Cystatin C. Overall, Cystatin C is not routinely used as a CVD biomarker (Sarnak, 2023).

Fibrinogen

Fibrinogen is a circulating glycoprotein that plays an important role in platelet aggregation and blood viscosity. Fibrinogen has been suggested as a possible indicator of inflammation that accompanies atherosclerosis. The independent predictive power, impact on management strategies, and clinical utility of fibrinogen measurement have shown conflicting results. One study of 150000 subjects demonstrated a log-linear relationship of fibrinogen and cardiovascular events, but another study of 90000 subjects did not find a relationship; therefore further research is required (Wilson, 2024b). A recent study has reported that higher fibrinogen levels increased the risk of a stroke in large arteries or small vessels but decreased the risk of cardioembolic stroke (Maners et al., 2019).

Pieters et al. (2020) investigated the contribution of fibrinogen, as well as other biomarkers, on cardiovascular disease (CVD) mortality. A total of 4487 patients were evaluated over a period of 14 years. The authors noted that 551 patients had CVD at baseline and over the time period investigated, 321 CVD deaths occurred. Fibrinogen was found to associate ("cluster") with C-Reactive protein only and was associated with both baseline CVD and CVD mortality at follow-up. Both fibrinogen and gamma-glutamyl transferase were found to be mediators between CVD status and all-cause mortality, as well as between CVD status and CVD mortality (Pieters et al., 2020).

Leptin

Leptin is a protein secreted by fat cells and plays a role in fat metabolism. As leptin increases with obesity, it is thought to be associated with CVD. Leptin may play a role in regulating blood pressure, insulin sensitivity, inflammatory vascular responses, and more. However, a meta-analysis covering 13 studies, 4257 CVD patients, and 26710 controls indicated no significant relationship between leptin and CVD, or stroke once other cardiovascular risk factors were controlled. The authors recommend further research to evaluate the effectiveness of leptin as a predictor of CVD (Wilson, 2024b; Yang et al., 2017). A recent study found that, in a Chinese cohort, serum leptin levels were identified as a marker for patients with first-ever acute ischemic stroke and were also associated with stroke size and severity (Liu et al., 2019).

Drug Therapies Requiring Lipid Monitoring

Lipid-lowering Therapy with Statins

Statins, such as ezetimibe, are a type of drug often prescribed to lower lipid levels or cholesterol. Pignone (2024) has reported that statins may reduce CVD risk by 20 to 30%, regardless of initial LDL-C levels. Statins are also beneficial for the treatment of arterial stiffness, independent of their hypolipidemic effect; treatment with a high dosage of statins will decrease LDL-C levels and improve arterial stiffness levels (Reklou et al., 2020). Kongpakwattana et al. (2019) report that the use of statin therapy in combination with non-stain lipid-modifying agents is more beneficial to reduce CVD risk than using only one treatment method.

A meta-analysis of statin trials completed by Boekholdt et al. (2014) analyzed data from 38,153 patients. During the follow-up of only 5,387 patients, it was found that 6,286 major cardiac events occurred. Great variability was recorded in LDL-C, apo B and non-HDL-C levels based on fixed statin levels over a one-year period. "Among trial participants treated with high-dose statin therapy, >40% did not reach an LDL-C target <70 mg/dl," suggesting that high-dose statin therapy effectiveness may depend on the individual (Boekholdt et al., 2014).

Antipsychotics

Several atypical antipsychotic medications, such as risperidone, sertindole and olanzapine, have been FDA approved for the treatment of psychiatric disorders, including bipolar disorder, depression, and schizophrenia; unfortunately, these medications may lead to a plethora of side effects, including dyslipidemia, hypertension, increased CVD risk, obesity, sudden cardiac death, and insulin resistance (Beauchemin et al., 2019; Polcwiartek et al., 2016). Specifically, antipsychotic-induced corrected QT prolongation may increase the risk of Torsades de Pointes (a form of polymorphic ventricular tachycardia), leading to sudden cardiac death (Polcwiartek et al., 2016). While newer antipsychotics have been improved to lessen the pro-arrhythmic impact of their predecessors, they may contribute to cardiac death in a new way: by worsening the metabolic profile (Howell et al., 2019). It is recommended that any individuals in need of antipsychotics seriously consider the risks of these medications before accepting this type of treatment.

A ten-year study compared the CVD risk of patients with schizophrenia taking antipsychotics with healthy controls. The overall CVD risk was 5.16% in patients with schizophrenia, and 3.02% in the healthy control group; further, risk scores were significantly higher and HDL levels were significantly lower in patients taking multiple antipsychotics (Kilicaslan et al., 2019). A recent meta-analysis by Rotella et al. (2020) aimed to identify the long-term metabolic and cardiovascular effects of antipsychotic drugs. A total of 3013 studies were screened, and 92 were used for data analysis. The researchers have found a

significantly higher risk of CVD death for sertindole users compared to risperidone users and state that "Long-term cardiovascular effects of APs [antipsychotics] deserve to be studied more extensively" (Rotella et al., 2020).

Accutane

Accutane, also known as isotretinoin, is a synthetic vitamin A derivative and oral medication often prescribed for the treatment of severe acne; it was approved by the FDA in 1982 to treat resistant, nodular acne that has not responded to conventional therapeutic measures such as systemic antibiotics (Pile & Sadiq, 2019). Unfortunately, isotretinoin therapy may cause various cardiac events, including congenital heart disease, atrial tachycardia, and cardiac remodeling (Güler et al., 2015). Akcay and Yuksel (2019) have reported that isotretinoin use may have been related to the development of Kounis syndrome (acute coronary syndrome due to a reduction of blood flow to the heart) in one patient. Alan et al. (2016) reported that isotretinoin use may have triggered premature ventricular contractions in a 33-year-old woman. Karadag et al. (2012) completed a study comprised of 70 patients who were being treated with 0.5-1.0 mg/kg per day of isotretinoin; in each patient, heart rate, blood pressure, EEG, biochemical and hematologic parameters were all measured. "We found that isotretinoin did not affect P- and QT-wave measurement" (Karadag et al., 2012).

Isotretinoin may also affect serum lipid levels. Zane et al. (2006) studied 13772 patients with acne currently receiving oral isotretinoin therapy. Results showed that 31% of isotretinoin users had high cholesterol levels, 11% had high liver transaminase levels, and 44% had high triglyceride levels (Zane et al., 2006). In a more recent study, Lee et al. (2016) completed a systematic review and meta-analysis from 1960-2013 which studied the effects of oral isotretinoin use. Data was only admitted if 40 mg/day of isotretinoin was used for at least four weeks. The authors stated that "This meta-analysis showed that (1) isotretinoin is associated with a statistically significant change in the mean value of several laboratory tests (white blood cell count and hepatic and lipid panels), yet (2) the mean changes across a patient group did not meet a priori criteria for high-risk and (3) the proportion of patients with laboratory abnormalities was low" (Lee et al., 2016; Zane et al., 2006). The authors concluded by stating that these results do not support monthly laboratory testing for patients taking standard isotretinoin doses for acne purposes.

Other Cardiovascular Markers

C Reactive Protein (CRP)

Data from numerous studies have shown an association between elevated serum or plasma concentrations of CRP and atherosclerotic vascular disease, risk of recurrent cardiovascular events, and the incidence of initial cardiovascular events among individuals not known to have atherosclerosis (Crea, 2023).

C Reactive Protein can be measured using either traditional assays or high sensitivity CRP (hs-CRP) assays. Traditional assays have limited use when screening for cardiovascular disease due to their limit of detection (3-5 mg/L). On the other hand, hs-CRP assays can detect concentrations of CRP down to 0.3 mg/L and below. These hs-CRP assays are used to assess cardiovascular risk because they can detect and quantify CRP within the range normally seen in asymptomatic patients (<3 mg/L). Elevated CRP levels, either alone or in combination with other cardiovascular risk factors, have been associated with a higher risk of future cardiovascular events. Studies evaluating CRP in asymptomatic populations have shown that the baseline level of CRP predicts the long-term risk of a first myocardial infarction (MI),

ischemic stroke, hypertension, peripheral vascular disease, sudden cardiac death, and all-cause mortality (Crea, 2023).

Homocysteine

Homocysteine is an amino acid that is produced by the body. Elevated levels of homocysteine may result in damage to the walls of the artery, increase the potential for thrombosis and lead to advanced atherosclerosis. Hence, elevated homocysteine levels have been demonstrated to increase the risk of CVD. However, the testing of homocysteine levels is not consistently recommended because, based on current research, the lowering of plasma homocysteine levels does not necessarily lower the risk of CVD. Further research is required to support the clinical utility of lowering homocysteine levels (R. S. Rosenson, Smith, C. Christopher, Bauer, Kenneth A., 2023).

Intermediate Density Lipoproteins (IDL)

Intermediate Density Lipoproteins (remnant cholesterol or lipoproteins) are the cholesterol content of triglyceride-rich lipoproteins, which is composed of VLDL and IDL in the fasting state, and is a combination of VLDL, IDL, and chylomicron remnants in the non-fasting state. It can be estimated by triglyceride (TG) levels in the absence of advanced lipoprotein testing. Elevated non-fasting plasma triglyceride is associated with increased risk for CVD (Varbo et al., 2013). Triglycerides are unlikely to directly cause CVD, thus VLDL and IDL are more commonly identified as the source of this increased risk for CVD (Jepsen et al., 2016). VLDL and IDL have been shown to be proatherogenic with both proinflammatory and prothrombotic effects (Joshi et al., 2016).

Genetic case studies have shown that elevated levels of remnant cholesterol are causally associated with both low-grade inflammation and CVD. Elevated levels of LDL cholesterol are associated with CVD, but not with low-grade inflammation. This indicates that elevated LDL cholesterol levels cause atherosclerosis without inflammation, whereas elevated remnant cholesterol levels lead to both atherosclerosis and inflammation (Varbo et al., 2014; Varbo et al., 2013).

Another measure which includes IDL is Non-HDL-C, which is derived from the simple calculation of total cholesterol minus HDL-C. The Emerging Risk Factors Collaboration concluded that apoB and non-HDL-C predicted risk similar to directly measured LDL-C and that fasting did not affect the hazard ratios (HRs) (Di Angelantonio et al., 2009).

Lipoprotein-associated Phospholipase A2 (Lp-PLA2)

Lipoprotein-associated phospholipase A2 (Lp-PLA2) is an inflammatory enzyme expressed in atherosclerotic plaques. It has been proposed that Lp– PLA2 testing may aid in detecting CVD risk due to its association with other biomarkers, such as LDL. The rationale for Lp-PLA2 as a key inflammatory biomarker is attractive because this enzyme is produced in atherosclerotic plaques with elevated expression found in CVD patients (Rosenson & Stafforini, 2012).

Numerous studies evaluate Lp-PLA2 as a predictor of cardiovascular risk (Garg et al., 2015; LPSC, 2010; Sudhir, 2006). These studies demonstrate that Lp-PLA2 is an independent predictor of CVD. Preliminary clinical trials of Lp-PLA2 inhibitors showed some improvements in physiologic measures, such as reduction in hs-CRP (Sudhir, 2006). However, further clinical trials of Lp-PLA2 inhibitors failed to demonstrate significant improvements in patient outcomes (Mohler et al., 2008). Although Lp-PLA2 does not appear to have any predictive power with apparently healthy individuals, it may have utility for symptomatic patients. The link between the enzyme and LDL is found in the enzyme's plasma activity,

which tends to vanish with treatment (Rosenson & Stafforini, 2012). De Stefano et al. (2019) stated that Lp-PLA2 may be considered as a new vascular specific biomarker to predict CVD in a population of patients with metabolic diseases.

Long-Chain Omega-3 Fatty Acids

Omega-3 fatty acids, a specific group of polyunsaturated fatty acids containing a double bond three carbons from the methyl terminus, are main building blocks of many fats and oils. Long-chain omega 3 fatty acids (≥C20, LC) include eicosapentaenoic acid (EPA, 20:5ω3), docosapentaenoic acid (DPA, 22:5ω3) and docosahexaenoic acid (DHA, 22:6ω3) and are thought to be beneficial in the prevention of coronary heart disease (CHD) (Mozaffarian, 2023). Circulating blood levels of EPA and DHA are inversely and significantly associated with reduced CHD event risk (de Oliveira Otto et al., 2013). Blood levels of omega-3 fatty acids may be more related to CVD benefit than the daily dose of fish oil supplements (Superko et al., 2014). The blood EPA/arachidonic acid (AA) ratio may be a clinically relevant measurement as AA has atherogenic and thrombogenic metabolites. Although this ratio has substantial individual variability, an EPA/AA ratio >0.75 has been associated with a significantly lower number of major coronary events in a Japanese population (Itakura et al., 2011). Determination of blood omega-3 levels may help guide the appropriate use of dietary fish or omega-3 supplements in a personalized heart disease prevention strategy.

The relationship of fish and dietary omega-3 fatty acids and cardiovascular disease (CVD) has been investigated in numerous studies and comprehensive reviews and recommendations exist, but guidance on blood concentrations is missing. Some prospective fish oil treatment investigations report a significant reduction in CVD events, but others do not (Bosch et al., 2012; Itakura et al., 2011). A meta-analysis did not find a statistically significant relationship between omega-3 consumption and CVD mortality (Rizos et al., 2012). A science advisory from the AHA stated that for individuals with prevalent CHD such as a recent MI event, treatment with omega-3 PUFA supplements is reasonable; further, for patients with prevalent heart failure without preserved left ventricular function, fish oil treatment is recommended, while treatment is not recommended for patients with diabetes mellitus, prediabetes or as a method for stroke prevention (Siscovick et al., 2017).

Troponins (I, T)

Troponins are specific biomarkers for cardiac injury and are often used to diagnose myocardial infarctions. These proteins control the calcium-mediated interaction of actin and myosin in the muscle, and the cardiac versions of these proteins are unique to the heart. There are two primary categories of tests for troponins; "sensitive or contemporary" and "high-sensitive." The high-sensitive version is preferred due to its superior accuracy (Gibson, 2024; Jaffe, 2024).

Elevated levels of troponins are proposed to predict CVD risk. Ford et al. (2016) performed a study evaluating troponin levels in 3318 men in relation to CVD risk. A hazard ratio of 2.3 for the highest quartile of troponin (≥5.2 ng/L) compared to the lowest quartile (≤3.1 ng/L) was found. The authors also found a 5-fold reduction in coronary events when troponin levels decreased by a quarter (Ford et al., 2016).

Tang et al. (2020) evaluated the ability of high-sensitivity cardiac troponin I (hs-cTnI) to assess cardiovascular risk and mortality. A total of 5876 patients ages 66-90 years were included. A total of 1053 deaths (321 CVD-related) occurred, within a median follow-up of 6.3 years. Patients with an elevated hs-cTnI and without history of CVD had a similar mortality risk to patients with a CVD history

but without an elevated hs-cTnI. However, after adjustment, elevated hs-cTnI was found to be associated with mortality risk, by a hazard ratio of 2.38 over low hs-cTnI and no CVD. Elevated hs-cTnI was found to be independently associated with incident CVD by a hazard ratio of 3.41, ASCVD (HR = 2.02) and heart failure (HR = 6.16). The authors concluded that "Hs-cTnI improves mortality and CVD risk stratification in older adults beyond traditional risk factors and improved model discrimination more than hs-cTnT for certain outcomes" (Tang et al., 2020).

Suthahar et al. (2020) "evaluate[d] associations of high-sensitivity cardiac troponin-T (cTnT) with cardiovascular disease (CVD), heart failure (HF), and mortality in community-dwelling women and men." A total of 8226 adults were included in the study. The authors detected cTnT levels in 1102 women and 2396 men. The authors found these baseline levels to be associated with a greater risk of developing CVD in women compared to men (women hazard ratio = 1.48, men hazard ratio = 1.20). Similar sex-related differences were found for heart failure and mortality. Women at six ng/L were also found to have significantly increased risk for CVD, HF, and mortality whereas men were only found to have significantly increased risk for CVD at the same level of cTnT (Suthahar et al., 2020).

Proprietary Testing

Cardiovascular Risk Panels/Profiles

Cardiovascular risk panels refer to combinations of cardiac markers that are used for the risk assessment of developing cardiovascular disease, major adverse cardiovascular events, or ischemic cerebrovascular events. Commercially available risk panels use different combinations of lipids, inflammatory, genetic, and metabolic markers. Risk panels report the results of multiple individual tests, whereas quantitative risk scores generally use proprietary algorithms to combine the results of multiple markers into one score. The clinical utility of risk panels is lacking as the impact of results on patient management is unknown.

Examples of commercially available cardiovascular risk panels include, but are not limited to:

1. Genova Diagnostics Cardio Check™ Panel:
    - Lipid markers (LDL; total cholesterol; HDL; triglycerides; lipoprotein (a); total cholesterol/HDL ratio; triglycerides/HDL ratio)
    - Independent risk factors (hs-CRP; homocysteine)
    - Insulin
    - Sex hormone markers (testosterone, total; sex hormone binding globulin) (Genova Diagnostics, 2023).
2. Cleveland HeartLab CVD Inflammation Testing Profile
    - F2-isoprostanes; oxidized LDL; hs-CRP; ADMA/SDMA; microalbumin; myeloperoxidase; Lp-PLA2 activity (HeartLab, 2023).
3. Thorne various packages:
    - Basic offerings include panels with markers such as Apo A-1 and Apo-B; the e-checkup comes with markers such as total cholesterol, HDL, LDL and Triglycerides, the basic panel adds additional biomarkers and the "premium" panel assesses 92 markers (Thorne, 2024).

Guidelines and Recommendations

American College of Cardiology (ACC) and the American Heart Association (AHA)

The 2019 ACC and AHA guidelines state that "Adults who are 40 to 75 years of age and are being

evaluated for cardiovascular disease prevention should undergo 10-year atherosclerotic cardiovascular disease (ASCVD) risk estimation and have a clinician–patient risk discussion before starting on pharmacological therapy, such as antihypertensive therapy, a statin, or aspirin. In addition, assessing for other risk-enhancing factors can help guide decisions about preventive interventions in select individuals, as can coronary artery calcium scanning" (Arnett et al., 2019).

Laboratory testing was not addressed in this update.

The ACC and AHA published joint guidelines on the assessment of cardiovascular risk in asymptomatic patients in 2010 (Greenland et al., 2010), and updated in 2013 (Goff et al., 2014).

In adults between the ages of 20 and 79 who are free from CVD, the ACC/AHA state that it is reasonable to assess risk factors (smoking, hypertension, diabetes, total cholesterol, high density lipoprotein cholesterol) every four to six years so as to calculate 10-year CVD risk (Goff et al., 2014).

The ACC/AHA also made the following recommendations on reclassification or contribution to risk assessment when high-sensitivity C-reactive protein (hs-CRP), apolipoprotein B (ApoB), glomerular filtration rate (GFR), microalbuminuria, family history, cardiorespiratory fitness, ankle-brachial index (ABI), carotid intima-media thickness (CIMT), or coronary artery calcium (CAC) score are considered in addition to the variables that are in the traditional risk scores:

1. If, after quantitative risk assessment, a risk-based treatment decision is uncertain, assessment of one or more of the following—family history, hs-CRP, ABI or CAC may be considered to inform treatment decision making.
2. CIMT is not recommended for routine measurement in clinical practice for risk assessment for a first ASCVD event.
3. The contribution to risk assessment for a first ASCVD event using ApoB, chronic kidney disease, albuminuria, or cardiorespiratory fitness is uncertain at present (Goff et al., 2014).

The 2010 guidelines contained the following statement concerning testing for Lp-PLA2: Lipoprotein-associated phospholipase A2 might be reasonable for cardiovascular risk assessment in intermediate-risk asymptomatic adults. However, the 2013 guidelines on the assessment of cardiovascular risk do not mention Lp-PLA2 testing (Goff et al., 2014; Greenland et al., 2010).

The updated guidelines do not address arterial compliance, lipoprotein-associated phospholipase, long-chain omega-3 fatty acids, or endothelial function assessment as methods to assess initial CVD risk (Goff et al., 2014; Greenland et al., 2010).

The ACC notes cutoffs of certain biomarkers for increased ASCVD risk, which are as follows: persistently elevated, primary hypertriglyceridemia ≥175 mg/dL, ≥2 mg/L hs-CRP, ≥50 mg/dL or ≥125 nmol Lp(a), ≥130 mg/dL Apo B (corresponding to >160 mg/dL LDL-C), and <0.9 ankle-brachial index (ABI) (ACC, 2018; Grundy et al., 2018).

The ACC and AHA also released joint guidelines with the AAPA, ABC, ACPM, AGS, APhA, ASH, ASPC, NMA, and PCNA, stating that screening and management of dyslipidemia/hypercholesterolemia is recommended in adults with hypertension (defined as >130/80 mmHg) (Whelton et al., 2018).

2018 AHA/ACC/AACVPR/AAPA/ABC/ACPM/ADA/AGS/APhA/ASPC/NLA/PCNA Guideline on the Management of Blood Cholesterol: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines

This joint report discusses management of blood cholesterol. The report addresses treatments, populations of interest, and serum assessments of relevant cardiovascular biomarkers such as Apo B and lipoprotein A. The relevant recommendations are listed below:

The report notes that although measurement of Apo B may be "unreliable," persistent elevation of Apo B may be considered a risk factor. The report remarks that a level of >130 mg/dL Apo B should be considered a risk-enhancing factor [of ASCVD], as it corresponds to an LDL-C level of ≥160 mg/dL.

The report also remarks that Lp(a) is considered a risk factor for ASCVD at levels of "≥50 mg/dL or ≥125 nmol/L." However, the authors write that it should be "considered in women only in the presence of hypercholesterolemia and with the understanding that the improvement in risk prediction in adult women in a large clinical trial was minimal."

Authors also recommend assessing "adherence and percentage response to LDL-C–lowering medications and lifestyle changes with repeat lipid measurement 4 to 12 weeks after statin initiation or dose adjustment, repeated every 3 to 12 months as needed."

The power of these risk factors can be seen in the "pooled cohort equation", "the single most robust tool for estimating 10-year risk in US adults 40 to 75 years of age." These algorithms have strong representative power for larger populations. However, a notable limitation of these algorithms is that they are not as accurate for individuals. Hence a clinician-patient risk discussion can individualize risk status based on PCE, but with the inclusion of additional risk-enhancing factors. These additional factors may include:

- "A family history of premature ASCVD (males, age <55 y; females, age <65 y)
- Primary hypercholesterolemia (LDL-C, 160–189 mg/dL [4.1–4.8 mmol/L); non–HDL-C 190–219 mg/dL [4.9–5.6 mmol/L])
- Metabolic syndrome (increased waist circumference, elevated triglycerides [>150 mg/dL], elevated blood pressure, elevated glucose, and low HDL-C [<40 mg/dL in men; <50 in women mg/dL] are factors; tally of 3 makes the diagnosis)
- Chronic kidney disease (eGFR 15–59 mL/min/1.73 m2 with or without albuminuria; not treated with dialysis or kidney transplantation)
- Chronic inflammatory conditions such as psoriasis, RA, or HIV/AIDS
- History of premature menopause (before age 40 y) and history of pregnancy-associated conditions that increase later ASCVD risk such as preeclampsia
- High-risk race/ethnicities (e.g., South Asian ancestry)
- Lipid/biomarkers: Associated with increased ASCVD risk
- Persistently elevated, primary hypertriglyceridemia (≥175 mg/dL)
- Elevated high-sensitivity C-reactive protein (≥2.0 mg/L)
- Elevated Lp(a): A relative indication for its measurement is family history of premature ASCVD. An Lp(a) ≥50 mg/dL or ≥125 nmol/L constitutes a risk-enhancing factor especially at higher levels of Lp(a)
- Elevated apoB ≥130 mg/dL: A relative indication for its measurement would be triglyceride ≥200 mg/dL. A level ≥130 mg/dL corresponds to an LDL-C ≥160 mg/dL and constitutes a risk-enhancing factor
- ABI <0.9" (Grundy et al., 2018)

American Diabetes Association (ADA)

The updated ADA Standards of Medical Care in Diabetes document also includes a section focused on cardiovascular disease and risk management. Relevant guidelines and notes are captured below.

- "Blood pressure should be measured at every routine clinical visit. When possible, individuals found to have elevated blood pressure (systolic blood pressure 120–129 mmHg and diastolic <80 mmHg) should have blood pressure confirmed using multiple readings, including measurements on a separate day, to diagnose hypertension."
- "All people with hypertension and diabetes should monitor their blood pressure at home ."
- "In asymptomatic patients, routine screening for coronary artery disease is not recommended as it does not improve outcomes as long as atherosclerotic cardiovascular disease risk factors are treated."
- "Consider investigations for coronary artery disease in the presence of any of the following: atypical cardiac symptoms (e.g., unexplained dyspnea, chest discomfort); signs or symptoms of associated vascular disease including carotid bruits, transient ischemic attack, stroke, claudication, or peripheral arterial disease; or electrocardiogram abnormalities."
- "Candidates for advanced or invasive cardiac testing include those with 1) typical or atypical cardiac symptoms and 2) an abnormal resting electrocardiogram (ECG). Exercise ECG testing without or with echocardiography may be used as the initial test. In adults with diabetes ≥40 years of age, measurement of coronary artery calcium is also reasonable for cardiovascular risk assessment. Pharmacologic stress echocardiography or nuclear imaging should be considered in individuals with diabetes in whom resting ECG abnormalities preclude exercise stress testing (e.g., left bundle branch block or ST-T abnormalities). In addition, individuals who require stress testing and are unable to exercise should undergo pharmacologic stress echocardiography or nuclear imaging."
- "The screening of asymptomatic patients with high ASCVD risk is not recommended, in part because these high-risk patients should already be receiving intensive medical therapy—an approach that provides benefit similar to invasive revascularization."
- "In adults not taking statins or other lipid-lowering therapy, it is reasonable to obtain a lipid profile at the time of diabetes diagnosis, at an initial medical evaluation, and every 5 years thereafter if under the age of 40 years, or more frequently if indicated."
- "…risk scores and other cardiovascular biomarkers have been developed for risk stratification of secondary prevention patients (i.e., those who are already high risk because they have ASCVD) but are not yet in widespread use."
- "The American College of Cardiology/American Heart Association ASCVD risk calculator (Risk Estimator Plus) is generally a useful tool to estimate 10-year risk of a first ASCVD event…The 10-year risk of a first ASCVD event should be assessed to better stratify ASCVD risk and help guide therapy, as described below."
- "Obtain a lipid profile at initiation of statins or other lipid-lowering therapy, 4–12 weeks after initiation or a change in dose, and annually thereafter as it may help to monitor the response to therapy and inform medication adherence" (ADA, 2020, 2021b, 2023).

Also, for children and adolescents, the following recommendations were given for dyslipidemia testing:

- "Initial lipid testing should be performed when initial glycemic control has been achieved and age is ≥2 years. If initial LDL cholesterol is ≤100 mg/dL (2.6 mmol/L), subsequent testing should be performed at 9-11 years of age. Initial testing may be done with a nonfasting non-HDL cholesterol level with confirmatory testing with a fasting lipid panel.

- If LDL cholesterol values are within the accepted risk level (<100 mg/dL [2.6 mmol/L]), a lipid profile repeated every 3 years is reasonable" (ADA, 2020, 2021a).

National Lipid Association (NLA)

The NLA published a scientific statement for lipid measurements in the management of cardiovascular disease, and their recommendations (with evidence rating of "B" or higher) are included below:

- "It is recommended to follow up abnormal screening lipid measurements with fasting lipid measurement (Strength: IIa. Evidence: B-NR)
- LDL-C in adults ≥ 190 mg/dL (≥160 mg/dl in children) is recommended to be reported as possible Familial Hypercholesterolemia (Strength: I. Evidence: B-NR)
- Non-HDL-C in adults ≥ 220 mg/dL is recommended to be reported as possible inherited hyperlipidemia (Strength: I. Evidence: B-NR)
- Triglyceride concentration ≥500 mg/dL is recommended to be reported as severe hypertriglyceridemia (Strength: I. Evidence: B-NR)
- Lipid measurements are recommended at 3-12 months for those on a stable medication regimen (Strength: I. Evidence: B-NR)
- Lipid measurements are recommended 4-12 weeks after a change in lipid treatment (Strength: I. Evidence: B-NR)
- LDL-C measurement is recommended for screening (Strength: I. Evidence: B-NR)
- LDL-C measurement is recommended on lipid therapy (Strength: I. Evidence: B-NR)
- Non-HDL-C measurement is recommended for screening (Strength: I. Evidence: B-NR)
- Non-HDL-C measurement is recommended on lipid therapy (Strength: I. Evidence: B-NR)
- Apolipoprotein B measurement may be reasonable for initial evaluation (Strength: IIb. Evidence: B-NR)
- Apolipoprotein B measurement is reasonable on lipid therapy (Strength: IIa. Evidence: B-NR)
- Apolipoprotein B measurement is recommended to facilitate diagnosis of Familial Dysbetalipoproteinemia and Familial Combined Hyperlipidemia (Strength: IIb. Evidence: B-NR)
- Lipoprotein (a) measurement is reasonable for initial evaluation in those with premature ASCVD, family history of premature ASCVD or of elevated Lp(a), history of LDL-C >190 mg/dL or suspected FH, or those with very high ASCVD risk (Strength: IIa. Evidence: B-NR)
- Lipoprotein (a) measurement may be reasonable on lipid therapy to determine those who may benefit from PCSK9 therapy who are already on maximal dose statin therapy ± ezetimibe, whose LDL-C remains above 70 mg/dl (Strength: IIb. Evidence: B-NR)"

Wilson et al. (2019) published a scientific statement to provide an update on the use of lipoprotein A [Lp(a)] in the clinical setting, particularly for atherosclerotic cardiovascular disease (ASCVD).

The Association lists the following recommendations for Lp(a) testing in clinical practice:

For adults over 20 years old, "Measurement of Lp(a) is **reasonable** to refine risk assessment for ASCVD events in:

- Individuals with a family history of first-degree relatives with premature ASCVD (<55 y[ears] of age in men, 65 y of age in women)
- Individuals with premature ASCVD (males aged <55 y and females aged <65 y), particularly in the absence of traditional risk factors
- Individuals with primary severe hypercholesterolemia (LDL ≥190 mg/dL) or suspected FH [familial

hypercholesterolemia]
- Individuals at very high** risk of ASCVD to better define those who are more likely to benefit from PCSK9 inhibitor therapy."
  **Very high risk is defined as "Individuals with a history of multiple major ASCVD events or 1 major ASCVD event and multiple high-risk conditions."

The guidelines further remark that "Measurement of Lp(a) **may be reasonable** with:

- Intermediate (7.5%–19.9%) 10-y ASCVD risk when the decision to use a statin is uncertain, to improve risk stratification in primary prevention.
- Borderline (5%–7.4%) 10-y ASCVD risk when the decision to use a statin is uncertain, to improve risk stratification in primary prevention.
- Less-than-anticipated LDL-C lowering, despite good adherence to therapy.
- A family history of elevated Lp(a).
- Calcific valvular aortic stenosis.
- Recurrent or progressive ASCVD, despite optimal lipid-lowering therapy."

Finally, the guidelines list recommendations for "youth" (<20 years old), stating that "Measurement of Lp(a) may be reasonable with:

- Clinically suspected or genetically confirmed FH.
- Individuals with a family history of first-degree relatives with premature ASCVD (<55 y of age in men, 65 y of age in women)
- An unknown cause of ischemic stroke
- A parent or sibling found to have an elevated Lp(a)" (Wilson et al., 2019).

A 2021 update was published, focused on practical and analytical recommendations (Wilson et al., 2021):

"Highlights include the following:

- It is acceptable to screen with nonfasting lipids.
- Non-high-density lipoprotein HDL-cholesterol (non-HDL-C) is measured reliably in either the fasting or the nonfasting state and can effectively guide ASCVD prevention.
- Low density lipoprotein cholesterol (LDL-C) can be estimated from total cholesterol, high density lipoprotein cholesterol (HDL-C), and triglyceride (TG) measurements. For patients with LDL-C>100 mg/dL and TG ≤150 mg/dL it is reasonable to use the Friedewald formula. However, for those with TG 150-400 mg/dL the Friedewald formula for LDL-C estimation is less accurate. The Martin/Hopkins method is recommended for LDL-C estimation throughout the range of LDL-C levels and up to TG levels of 399 mg/dL. For TG levels ≥400 mg/dL LDL-C estimating equations are currently not recommended and newer methods are being evaluated.
- When LDL-C or TG screening results are abnormal the clinician should consider obtaining fasting lipids.
- Advanced lipoprotein tests using apolipoprotein B (apoB), LDL Particle Number (LDL-P) or remnant cholesterol may help to guide therapeutic decisions in select patients, but data are limited for patients already on lipid lowering therapy with low LDL-C levels. Better harmonization of advanced lipid measurement methods is needed. Lipid measurements are recommended 4-12 weeks after a change in lipid treatment. Lipid laboratory reports should denote desirable values and specifically identify extremely elevated LDL-C levels (≥190 mg/dL at any age or ≥160 mg/dL in children) as severe hypercholesterolemia. Potentially actionable abnormal lipid test results,

including fasting triglycerides (TG) ≥500 mg/dL, should be reported as hypertriglyceridemia. Appropriate use and reporting of lipid tests should improve their utility in the management of persons at high risk for ASCVD events."

Centers for Disease Control and Prevention (CDC)

The CDC highlights the importance of cardiovascular disease biomarkers and has developed a reference laboratory and clinical standardization program to provide reference measurements for HDL-C, LDL-C, TG and total cholesterol (TC). The accuracy of the labs that analyze these biomarkers is also monitored by the CDC (CDC, 2024a).

The CDC notes that several health conditions increase the risk of heart disease including smoking, diabetes mellitus, obesity, high blood pressure, excessive alcohol use, physical inactivity, and unhealthy blood cholesterol levels. It is stated that "High blood cholesterol usually has no signs or symptoms. The only way to know whether you have high cholesterol is to get your cholesterol checked. Your health care team can do a simple blood test, called a "lipid profile," to measure your cholesterol levels (CDC, 2024b).

The CDC has also developed the Lipids Standardization Program (LSP). This program ensures that the measurements reported in research studies and clinical laboratories are accurate. Blinded samples traceable to the CDC Reference Laboratory are provided to participants. The samples will be measured for total cholesterol (TC), glycerides (TG), high-density lipoprotein cholesterol (HDL-C), apolipoprotein A-I (apo A-I), and apolipoprotein B (apo B). LSP participants report their results from the provided samples back to the CDC where these results are then analyzed; if results are accurate, those laboratories receive a certificate and are considered CDC-certified (CDC, 2023).

Centers for Disease Control and Prevention (CDC)/American Heart Association (AHA)

In 2002, the CDC and AHA held the "CDC/AHA Workshop on Inflammatory Markers and Cardiovascular Disease: Applications to Clinical and Public Health Practice" and released these recommendations in 2003. In this workshop, they looked at evidence surrounding multiple inflammatory markers and based on all considerations, they stated that "it is most reasonable to limit current assays of inflammatory markers to hs-CRP, measured twice, either fasting or nonfasting, with the average expressed in mg/L, in metabolically stable patients." More specifically, they indicate that the two measurements of hs-CRP should optimally occur two weeks apart. If results are abnormal, hs-CRP testing should be repeated and the patient should be examined for sources of infection or inflammation. Furthermore, they provide the following recommendation specific to hs-CRP: "On the basis of the available evidence, the Writing Group recommends against screening of the entire adult population for hs-CRP as a public health measure. The Writing Group does conclude that it is reasonable to measure hs-CRP as an adjunct to the major risk factors to further assess absolute risk for coronary disease primary prevention. At the discretion of the physician, the measurement is considered optional, based on the moderate level of evidence (Evidence Level C). In this role, hs-CRP measurement appears to be best employed to detect enhanced absolute risk in persons in whom multiple risk factor scoring projects a 10-year CHD risk in the range of 10% to 20% (Evidence Level B). However, the benefits of this strategy or any treatment based on this strategy remain uncertain. The finding of a high relative risk level of hs-CRP (>3.0 mg/L) may allow for intensification of medical therapy to further reduce risk and to motivate some patients to improve their lifestyle or comply with medications prescribed to reduce their risk. Individuals at low risk (<10% per 10 years) will be unlikely to have a high risk (>20%) identified through hs-CRP testing. Individuals at high risk (>20% risk over 10 years) or with established atherosclerotic disease generally should be treated intensively regardless of their hs-CRP levels, so the utility of hs-CRP in secondary

prevention appears to be more limited" (Pearson et al., 2003).

Centers for Medicare & Medicaid Services (CMS)

In 2005, CMS published national coverage determination that discuss appropriate frequency of monitoring for individuals receiving long term pharmacologic treatments that may affect lipid levels and thus may affect that individuals risk of developing CVD. The indications and limitations of coverage state:

"When monitoring long term anti-lipid dietary or pharmacologic therapy and when following patients with borderline high total or LDL cholesterol levels, it is reasonable to perform the lipid panel annually. A lipid panel at a yearly interval will usually be adequate while measurement of the serum total cholesterol or a measured LDL should suffice for interim visits if the patient does not have hypertriglyceridemia" (CMS, 2005).

"Any one component of the panel or a measured LDL may be medically necessary up to six times the first year for monitoring dietary or pharmacologic therapy. More frequent total cholesterol HDL cholesterol, LDL cholesterol and triglyceride testing may be indicated for marked elevations or for changes to anti-lipid therapy due to inadequate initial patient response to dietary or pharmacologic therapy. The LDL cholesterol or total cholesterol may be measured three times yearly after treatment goals have been achieved" (CMS, 2005).

"If no dietary or pharmacological therapy is advised, monitoring is not necessary" (CMS, 2005).

American Heart Association (AHA)

In 2022, the AHA published a scientific statement to highlight the history, biology, pathophysiology, and emerging clinical evidence in the lipoprotein a (Lp[a]) field, where they addressed the "key knowledge gaps and future directions required to mitigate the atherosclerotic cardiovascular disease risk attributable to elevated Lp(a) levels." In this statement, they noted that "levels of Lp(a) have not been shown to substantially change across the life course, although some variability occurs, as documented by intraindividual temporal variability in serial measurements from placebo-treated subjects in clinical trials." They also note that "various organizations have proposed to obtain a level once in every adult" (Reyes-Soffer et al., 2022).

American Association of Clinical Endocrinologists (AACE) and American College of Endocrinology (ACE)

The 2017 AACE and ACE Guidelines for Management of Dyslipidemia and Prevention of Cardiovascular Disease recommend:

- Screening guidelines for dyslipidemia vary by age group;
- Although ASCVD risk in young adults is low, adults older than 20 years should be evaluated for dyslipidemia every five years as part of a global risk assessment
- Middle-aged individuals (Men 45-65 years, Women 55-65 years) should be screened for dyslipidemia at least every one to two years.
- All individuals with diabetes should be screened with a lipid profile at the time of diagnosis and annually thereafter. Some individuals with diabetes can be screened less frequently based on clinical considerations
- Annual screen for dyslipidemia for adults over 65 is recommended

- In children at risk for FH (e.g., family history of premature cardiovascular disease or elevated cholesterol), screening should be at three years of age, between 9 and 11, and at age 18
- Screen adolescents older than 16 years every five years or more frequently if they have ASCVD risk factors, have overweight or obesity, have other elements of the insulin resistance syndrome, or have a family history of premature ASCVD
- Direct measurement of LDL-C should be used to assess LDL-C in certain high-risk individuals, such as those with fasting TG concentrations greater than 250 mg/dL or those with diabetes or known vascular disease
- Apolipoproteins, Apo B and/or an apo B/apo A1 ratio calculation and evaluation may be useful in at-risk individuals.
- hsCRP is recommended to stratify ASCVD risk in individuals with a standard risk assessment that is borderline, or in those with an intermediate or higher risk with an LDL-C concentration <130 mg/dL.
- Lp-PLA2 measurement, is recommended when it is necessary to further stratify an individual's ASCVD risk, especially in the presence of hsCRP elevations.
- The routine measurement of homocysteine, uric acid, plasminogen activator inhibitor-1, or other inflammatory markers is not recommended because the benefit of doing so is not sufficiently proven.
- Coronary artery calcification measurement has been shown to be of high predictive value and is useful in refining risk stratification
- Carotid intima media thickness (CIMT) may be considered to refine risk stratification (Jellinger et al., 2017).

The AACE/ACE published an updated algorithm in 2020. This algorithm focuses on "management of dyslipidemia and prevention of cardiovascular disease" and "complements" the above guidelines but includes information not available in 2017. Their relevant recommendations are listed below:

The guideline lists Apo B, LDL, Lp(a), and hs-CRP as biomarkers that may be "considered" in assessment of ASCVD risk for patients. The guideline also remarks that "measurement of apo B is useful in assessing the success of lipid-lowering therapy, since apo B may remain above goal after achieving the LDL-C goal." Apo B is listed as a component of treatment goals, alongside LDL-C, Non-HDL-C, and TG [triglycerides].

The guideline recommends "considering" measurement of Lp(a) (lipoprotein A) in the following settings:

- "All patients with clinical ASCVD, especially premature or recurrent ASCVD despite LDL-C lowering;
- Individuals with a family history of premature ASCVD and/or increased Lp(a);
- Individuals with South Asian or African ancestry, especially with a family history of ASCVD or increased Lp(a);
- Individuals with a 10-year ASCVD risk ≥10% (primary prevention setting), in order to stratify risk;
- Patients with a personal or family history of aortic valve stenosis;
- Patients with refractory elevations of LDL-C despite aggressive LDL-C-lowering therapy (i.e., statin resistance)" (AACE, 2021).

The AACE also published a "consensus statement" on the "comprehensive type 2 diabetes management algorithm". The guideline includes a set of PowerPoint slides at the bottom, which recommend measuring Lp(a) in the following settings: presence of family history of premature ASCVD and/or increased Lp(a), and all patients with premature or recurrent ASCVD despite LDL-C lowering (Garber et

al., 2020).

European Society of Cardiology (ESC) and European Atherosclerosis Society (EAS) Guidelines for the Management of Dyslipidaemias

The ESC published 2021 guidelines on cardiovascular disease prevention in clinical practice. Their recommendations for CVD risk assessment are included below (Visseren et al., 2021):

Recommendations	Class[a]	Level[b]
Systematic global CVD risk assessment is recommended in individuals with any major vascular risk factor (i.e. family history of premature CVD, FH, CVD risk factors such as smoking, arterial hypertension, DM, raised lipid level, obesity, or comorbidities increasing CVD risk).	I	C
Systematic or opportunistic CV risk assessment in the general population in men >40 years of age and in women >50 years of age or postmenopausal with no known ASCVD risk factors may be considered.[9]	IIb	C
In those individuals who have undergone CVD risk assessment in the context of opportunistic screening, a repetition of screening after 5 years (or sooner if risk was close to treatment thresholds) may be considered.	IIb	C
Opportunistic screening of BP in adults at risk for the development of hypertension, such as those who are overweight or with a known family history of hypertension, should be considered.[19]	IIa	B
Systematic CVD risk assessment in men <40 years of age and women <50 years of age with no known CV risk factors is not recommended.[9]	III	C

The authors include additional recommendations for CVD risk estimation and modification that are

tabulated in the full guideline.

The 2019 guidelines from ESC and EAS provided the following recommendations:

- "Lp(a) measurement should be considered at least once in each adult person's lifetime to identify those with very high inherited Lp(a) levels >180 mg/dL (>430 nmol/L) who may have a lifetime risk of ASCVD equivalent to the risk associated with heterozygous familial hypercholesterolemia.
- "Persons with documented ASCVD, type 1 or type 2 DM (T1DM and T2DM, respectively), very high levels of individual risk factors, or chronic kidney disease (CKD) are generally at very-high or high total CV risk. No risk estimation models are needed for such persons…"
- ApoB analysis is recommended for risk assessment, particularly in people with high TG, DM, obesity or metabolic syndrome, or very low LDL-C. It can be used as an alternative to LDL-C, if available, as the primary measurement for screening, diagnosis, and management, and may be preferred over non-HDL-C in people with high TG, DM, obesity, or very low LDL-C.
- CAC score assessment with CT may be helpful in reaching decisions about treatment in people who are at moderate risk of ASCVD. Obtaining such a score may assist in discussions about treatment strategies in patients where the LDL-C goal is not achieved with lifestyle intervention alone and there is a question of whether to institute LDL-C-lowering treatment. Assessment of arterial (carotid or femoral) plaque burden on ultrasonography may also be informative in these circumstances" (Mach et al., 2019).

Total cholesterol may be used to estimate total cardiovascular risk. LDL-C is recommended to be used as the primary lipid analysis for diagnosis, management, screening, and risk estimation. HDL-C and Non-HDL-C are also strong, independent risk factors (Catapano et al., 2016).

Apolipoprotein B , Lp(a), Apo B/Apo A-I, and Non-HDL-C/HDL-C may all be used as alternative markers for cardiovascular risk. The guidelines note that measuring Apo B and Apo A-I is convenient, accurate, does not require fasting, and is not susceptible to TG levels. The guidelines also recommend against routine measurement of Apo C-III as its use is unknown (Catapano et al., 2016).

In the 2021 ESC Guidelines on cardiovascular disease prevention, the authors stated that "New studies confirm that C-reactive protein has limited additional value. There is renewed interest in lipoprotein(a), but it too provides limited additional value in terms of reclassification potential. Cardiac biomarkers are promising, but further work is needed" (Visseren et al., 2021).

European Society of Cardiology (ESC) and the European Association for the Study of Diabetes (EASD)

This joint guideline was published for "diabetes, pre-diabetes, and cardiovascular diseases". Their specific recommendations relating to cardiovascular risk assessment in patients with diabetes and pre-diabetes are as follows:

- "Routine assessment of microalbuminuria should be carried out to identify patients at risk of developing renal dysfunction and/or CVD."
- "A resting electrocardiogram (ECG) is indicated in patients with DM and hypertension, or if CVD is suspected."
- "Other tests, such as transthoracic echocardiography, coronary artery calcium (CAC) score, and ankle–brachial index (ABI), may be considered to test for structural heart disease or as risk modifiers in those at moderate or high risk of CVD."
- "Routine assessment of novel biomarkers is not recommended for CV risk stratification."

The guideline noted that "the addition of circulating biomarkers for CV risk assessment has limited clinical value" and stated that "in patients with DM [diabetes mellitus] without known CVD, measurement of C-reactive protein or fibrinogen (inflammatory markers) provides minor incremental value to current risk assessment". Cosentino et al. (2020) guideline also noted high-sensitivity cardiac troponin T as not adding incremental "discriminative power" for patients with DM without known CVD, although elevated high-sensitivity cardiac troponin T was noted as an independent predictor of renal decline and CV events in patients with type 1 diabetes (Cosentino et al., 2020)

Endocrine Society (ES)

This guideline was published with the intent to assess and treat dyslipidemia in patients with endocrine disorders. Their relevant recommendations are listed below:

- "In adults with endocrine disorders, we recommend a lipid panel for the assessment of triglyceride levels and for calculating low-density lipoprotein cholesterol."
- "In adults with endocrine disorders, we recommend conducting a cardiovascular risk assessment by evaluating traditional risk factors, including the calculation of 10-year atherosclerotic cardiovascular disease risk using a tool such as the Pooled Cohort Equations."
- "In adults with endocrine disorders at borderline or intermediate risk (10-year atherosclerotic cardiovascular disease risk 5%–19.9%), particularly those with additional risk-enhancing factors, in whom the decision about statin treatment and/or other preventive interventions is uncertain, we suggest measuring coronary artery calcium to inform shared decision making."

The guideline also remarks that certain "advanced" lipid testing (assessment of markers such as Apo B, lipid fractionation, and Lp(a)) may be helpful in "characterizing" lipid abnormalities, but "add little" to risk prediction beyond the standard lipid profile.

The guideline goes on to discuss Lp(a), noting that the marker can be helpful in assessing familial risk, but adds "little" in terms of global risk assessment across the general population. The guideline acknowledges other evidence supporting Lp(a)'s use as a marker to manage treatment. Other serum biomarkers and biomarker panels were also considered to add "little" to global risk assessment. Finally, the guideline recommends the use of hs-CRP as a "risk-enhancing factor that may drive more aggressive treatment or the need for advanced risk assessment" (Newman et al., 2020).

Rosenzweig et al. (2019) published on the Primary Prevention of ASCVD and T2DM in Patients at Metabolic Risk. A summary of the recommendations is included below (Rosenzweig et al., 2019):

- "In individuals aged 40–75 years in the office setting, we suggest providers screen for all five components of metabolic risk at the clinical visit. The finding of at least three components should specifically alert the clinician to a patient at metabolic risk (at higher risk for atherosclerotic cardiovascular disease and type 2 diabetes mellitus).
- In individuals aged 40-75 years in the office setting who do not yet have atherosclerotic cardiovascular disease or type 2 diabetes mellitus and already have at least one risk factor, we advise screening every 3 years for all five components of metabolic risk as part of the routine clinical examination.
- To establish metabolic risk in the general population, we recommend that clinicians measure waist circumference as a routine part of the clinical examination.
- In individuals previously diagnosed with prediabetes, we suggest testing at least annually for the presence of overt type 2 diabetes mellitus.

- We recommend that all individuals at metabolic risk in the office setting have their blood pressure measured annually and, if elevated, at each subsequent visit.
- For individuals with elevated blood pressure above 130 mmHg systolic and/or 80 mmHg diastolic who are not documented as having a history of hypertension, we recommend confirmation of elevated blood pressure on a separate day within a few weeks or with a home blood pressure monitor."

Components of "metabolic risk" are defined as:

- elevated blood pressure
- increased waist circumference
- elevated fasting triglycerides
- low high-density lipoprotein-cholesterol, and
- elevated glycemia

American Society for Clinical Pathology

The ASCP recommends against routinely ordering expanded lipid panels (such as particle sizing or nuclear magnetic resonance) as screening for cardiovascular disease (ASCP, 2016).

In 2022, the ASCP published a set of clinical recommendations aiming to provide the guidance and the tools for assessment of ASCVD risk with the goal of appropriately targeting treatment approaches for prevention of ASCVD events, as shown below:

"1 Assessing a patient's risk for ASCVD is the foundation of preventive cardiology and the initial step for determining the appropriateness and intensity of preventive treatment.

2 In primary prevention, global risk scoring is the initial stage for ASCVD risk assessment, providing a calculation of ASCVD risk from a set of standard office-based risk factors for a specified duration (e.g., 10 years) of time, from which a clinician-patient risk discussion is used to discuss the best ways to reduce CVD risk.

3 The presence, quantity, and/or extent of one or more risk enhancing factors, including premature family history, persistently elevated LDL-C, or CKD, as well as severity of certain inflammatory factors such as hsCRP and laboratory measures such as lp(a), can further inform the treatment decision.

4 In women, it is important to take a comprehensive reproductive history from menarche to menopause, including preeclampsia, premature menopause, and autoimmune disease as "risk-enhancing" factors.

5 Race/ethnicity may have a significant impact on the validity of current risk assessment tools and certain higher risk race/ethnic groups may further inform the use of preventive therapy.

6 Social determinants of health may exert independent effects beyond race/ethnicity and need also to be part of the clinician-patient discussion when discussing the most appropriate ways to optimize ASCVD risk.

7 Among subclinical atherosclerotic disease screening tests, CAC is probably the most useful, providing substantial improvement of risk reclassification over global risk scoring in most primary prevention groups, including diabetes. In addition to the consideration of risk enhancing factors (discussed earlier),

CAC testing can be used to further inform treatment decisions for preventive therapy, including statin and aspirin use in particular.

8 The use of ABI for assessment of PAD is also valuable and can improve risk reclassification beyond global risk scoring.

9 Carotid ultrasound imaging, if accompanied by carotid plaque assessment may also be useful for risk assessment, especially as an option when CAC scoring is not available.

10 In patients with pre-existing ASCVD, stratification into those at highest risk (e.g., very high risk ASCVD status) for more aggressive treatment is based on the history of multiple major ASCVD events or one major event and multiple high-risk conditions. Moreover, those with recurrent ASCVD events in the short-term define an extreme risk condition warranting even more aggressive risk factor management" (Wong et al., 2022).

National Institute for Health and Care Excellence (NICE)

A baseline lipid profile should be taken before treatment. This should include total cholesterol, HDL cholesterol, non-HDL, and triglyceride levels. Total and HDL cholesterol should be measured for best estimate of CVD risk.

Omega-3 compounds have "no evidence" to help prevent CVD and NICE recommends against distribution of these compounds for CVD treatment (NICE, 2023).

US Preventive Services Task Force (USPSTF)

The USPSTF Task Force Recommendations include periodic assessment of cardiovascular risk factors from ages 40 to 75 years, including measurement of total cholesterol, LDL-C, and HDL-C levels. The optimal intervals for cardiovascular risk assessment are uncertain. Based on other guidelines and expert opinion, reasonable options include annual assessment of blood pressure and smoking status and measurement of lipid levels every five years. Shorter intervals may be useful for persons at higher risk, and longer intervals are appropriate for persons who are regularly at average risk (Bibbins-Domingo et al., 2017).

The USPSTF found insufficient evidence that screening for dyslipidemia in younger adults influences cardiovascular outcomes, and no studies that evaluated the effects of screening vs no screening, treatment vs no treatment, or delayed vs earlier treatment in adults in this age group. Thus, the USPSTF recommends neither for nor against screening for dyslipidemia in this age group. The USPSTF also noted there was insufficient evidence to assess the balance of benefits and harms of screening for dyslipidemia in children and adolescents (Chou et al., 2016).

The USPSTF states that "current evidence is insufficient to assess the benefits and harms of adding ankle-brachial index (ABI), high-sensitivity C-reactive protein (hsCRP) level, or coronary artery calcium (CAC) score to traditional risk assessment for cardiovascular disease (CVD) in asymptomatic adults to prevent CVD events" (USPSTF, 2018a). However, the USPSTF recommends screening for abnormal blood glucose for adults aged 40-70 who are overweight or obese (USPSTF, 2015).

For adults at low risk of CVD events, "The USPSTF recommends against screening with resting or exercise electrocardiography (ECG) to prevent cardiovascular disease (CVD) events" (recommendation grade D, discouraging the use of the service). Moreover, "The USPSTF concludes that the current

evidence is insufficient to assess the balance of benefits and harms of screening with resting or exercise ECG to prevent CVD events in asymptomatic adults at intermediate or high risk of CVD events" (recommendation grade I, insufficient evidence) (USPSTF, 2018b).

The USPSTF concludes that the current evidence is insufficient to assess the balance of benefits and harms of screening for primary hypertension in asymptomatic children and adolescents to prevent subsequent cardiovascular disease (Moyer, 2013). A 2020 recommendation statement by the USPSTF confirmed that the current evidence is insufficient to assess the balance of benefits and harms of screening for high blood pressure in children and adolescents (in general) (USPSTF, 2020). In adults, however, The USPSTF recommends screening for hypertension (persons 18 years or older) with office blood pressure measurement (OBPM). The USPSTF recommends obtaining blood pressure measurements outside of the clinical setting for diagnostic confirmation before starting treatment (USPSTF, 2021).

Finally, screening for obesity in children six years or older is recommended (Bibbins-Domingo et al., 2017).

Canadian Cardiovascular Society (CCS)

In 2021, the CCS published updated their recommendations on the management of dyslipidemia for the prevention of cardiovascular disease in adults. A summary of the society's recommendations that are relevant to CVD risk assessment is provided below:

- "Among women whose pregnancy was complicated by the hypertensive disorders of pregnancy -- gestational hypertension and/or preeclampsia -- or a preterm birth before 34 weeks' gestation, a stillbirth and/or a placental abruption, we recommend screening with a comprehensive lipid panel at least 12 weeks postpartum. These women have a higher risk of premature CVD and stroke within 10-15 years after the affected pregnancy (Strong Recommendation, Moderate Quality Evidence).
- To assist with decisions about initiating lipid-lowering pharmacotherapy in a nonpregnant woman who had one or more of these pregnancy complications, we recommend referral to a specialized postpartum cardiovascular health clinic or specialized lipid clinic, if locally available. If such resources are not locally available, we recommend using standard risk assessment tools to decide about lipid-lowering pharmacotherapy. However, when interpreting their 10-year CVD risk using a risk calculator, it is important to note that most women in this group will be found to have a low calculated absolute risk of CVD, short-term, which may give a false sense of reassurance to both the patient and her health care provider. (Weak Recommendation; Low-Quality Evidence).
- For any patient with triglycerides > 1.5mM, use non-HDL-C or apoB instead of LDL-C as the preferred lipid parameter for initial screening and treatment target (< 2.6 mM for non-HDL-C or < 0.8 g/L for apoB) in intermediate or high risk individuals (Strong Recommendation, High-Quality Evidence).
- We recommend measuring lipoprotein (a) level once in a person's lifetime as a part of the initial lipid screening (Strong Recommendation; High-Quality Evidence).
- We suggest that CAC [coronary artery calcium] screening using computed tomography imaging might be considered for asymptomatic adults 40 years or older and at intermediate risk (FRS 10%-20%) for whom treatment decisions are uncertain (Strong Recommendation, Moderate-Quality Evidence).
- We recommend that CAC screening using computed tomography imaging not be undertaken for: (1) high-risk individuals; (2) patients receiving statin treatment; or (3) most asymptomatic, low-risk

adults (Strong Recommendation; Moderate-Quality Evidence).
- We suggest that CAC screening might be considered for a subset of low-risk individuals 40 years or older (Weak Recommendation; Low-Quality Evidence)" (Pearson et al., 2021).

The 2021 guidelines affirmed those from 2016, stating that "Screening should be repeated every 5 years for men and women aged 40-75 years using the modified FRS or Cardiovascular Life Expectancy Model (CLEM) to guide therapy to reduce major CV events."

A revision of the 2016 recommendation is the role of Lp(a): "Lp(a) is not currently considered a treatment target and repeat measures are therefore not indicated." Moreover, non-fasting lipid testing is recommended during the CV risk assessment, and "It is now generally preferable to follow non-HDL-C or ApoB levels over LDL-C when interpreting lipid results, particularly when TG are ≥ 1.5 mmol/L" (Pearson et al., 2021).

HIV Medicine Association of the Infectious Diseases Society of America

HIV-infected patients commonly develop dyslipidemia after starting antiretroviral therapy (ART). The lipid abnormalities developed in HIV-infected patients are associated with increased cardiovascular risk. HIV Medicine Association of the Infectious Diseases Society of America have updated their guidelines in 2013 to include a new section on metabolic comorbidities. They recommend obtaining a fasting lipid profile prior to and within 1-3 months after starting ART and every 6-12 months in all patients. The 2020 update affirms this, as under the workup for routine healthcare maintenance considerations for persons with HIV, they recommend the following steps: "Lipid profile: perform every 5 years if normal; more frequently if abnormal or other cardiovascular risk factors present (every 6–12 months); if abnormal, repeat fasting" and ask that clinicians "Follow the atherosclerotic cardiovascular disease risk calculator. Consider testing 1–3 months after starting or changing ART" (Thompson et al., 2020).

The Association also notes that HbA1c may be tested or used for screening and states that a lower cutoff of 5.8% for diabetes may be used for patients on ART instead of the higher 6.5%. Finally, the Association recommends measuring HbA1c every six months in patients with diabetes (Aberg et al., 2014; Thompson et al., 2020).

According to the Association, an initial evaluation and an immediate follow-up for persons with HIV includes "A comprehensive present and past medical history that includes HIV-related information, medication/social/family history…, review of systems, and physical examination… should be obtained for all patients upon initiation of care, ideally at the first visit or, if not feasible, as soon as possible thereafter," and this includes testing for "Cardiovascular disease and risk factors, including hyperlipidemia, hypertension, diabetes mellitus, smoking" (Thompson et al., 2020).

U.S. Department of Veterans Affairs (VA) and U.S. Department of Defense (DoD)

The United States VA and DoD published a joint guideline regarding management of dyslipidemia for reducing CVD risk in adults. Their relevant recommendations are listed below:

- "For primary prevention in patients over age 40 and not on statin therapy who have not developed new cardiovascular risk factors (e.g., diabetes, hypertension, tobacco use), we suggest against offering a cardiovascular disease risk assessment more frequently than every five years."
- "For primary prevention in patients not on statin therapy, we suggest against routinely ordering a lipid panel more frequently than every 10 years."
- "For cardiovascular risk assessment in primary prevention, we suggest using a 10-year risk

calculator."
- "We suggest against the routine use of coronary artery calcium testing."
- "We suggest against the routine use of additional risk markers (e.g., high-sensitivity C-reactive protein, ankle-brachial index, coronary artery calcium) when assessing cardiovascular risk."

The guideline also remarks that several other markers, such as "coronary artery calcium (CAC), high-sensitivity C-reactive protein, ankle–brachial index, and apolipoprotein evaluations" have been proposed as useful tools to determine risk. However, these markers have been deemed "limited in further refining risk." Although CAC was considered to best of the markers listed, the guideline still recommended against routine CAC testing.

The guideline also recommends against "routine lipid level testing for risk assessment and monitoring, unless it is specifically intended to guide decision making" (O'Malley et al., 2020).

2021 European Society of Cardiology (ESC) and Other Societies on Cardiovascular Disease Prevention in Clinical Practice

In 2021, the Seventh Joint Task Force of the ESC and Other Societies on Cardiovascular Disease Prevention in Clinical Practice published guidelines on cardiovascular disease prevention in clinical practice.

The 2021 update has the same goals and targets for LDL-C, BP, and glycemic control in patients with diabetes mellitus as the previous 2016 guideline.

The authors state that routine assessment of circulating or urinary biomarkers is still not recommended for CVD risk stratification. The Task Force states that there is conflicting data on the utility of these biomarkers (such as hsCRP, various apolipoproteins, etc.) and that new studies confirm that C-reactive protein has "limited additional value." The authors concede that there is renewed interest in lipoprotein a, but that it doesn't add additional value in risk stratification. While cardiac biomarkers show promise, additional clinical studies are necessary.

The Task Force recommends repeating risk assessment every five years, and more often for higher risk patients. However, the Task Force only recommends this screening procedure for men >40 years and women >50 years, declaring that, though it is not cost-effective, a systematic CVD risk assessment is shown to "increase detection of CV risk factors" (Visseren et al., 2021).

Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are

not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
81599	Unlisted multianalyte assay with algorithmic analysis
82172	Apolipoprotein, each
82465	Cholesterol, serum or whole blood, total
82610	Cystatin C
83090	Homocysteine
83695	Lipoprotein (a)
83698	Lipoprotein-associated phospholipase A2 (Lp-PLA2)
83700	Lipoprotein, blood; electrophoretic separation and quantitation
83701	Lipoprotein, blood; high resolution fractionation and quantitation of lipoproteins including lipoprotein subclasses when performed (eg, electrophoresis, ultracentrifugation)
83704	Lipoprotein, blood; quantitation of lipoprotein particle number(s) (eg, by nuclear magnetic resonance spectroscopy), includes lipoprotein particle subclass(es), when performed
83718	Lipoprotein, direct measurement; high density cholesterol (HDL cholesterol)
83719	Lipoprotein, direct measurement; VLDL cholesterol
83721	Lipoprotein, direct measurement; LDL cholesterol
83722	Lipoprotein, direct measurement; small dense LDL cholesterol
83880	Natriuretic peptide
84478	Triglycerides
84484	Troponin, quantitative
84512	Troponin, Qualitative
84999	Unlisted chemistry procedure
85384	Fibrinogen; activity
85415	Fibrinolytic factors and inhibitors; plasminogen activator
86140	C-reactive protein
86141	C-reactive protein; high sensitivity (hsCRP)
0019M	Cardiovascular disease, plasma, analysis of protein biomarkers by aptamer based microarray and algorithm reported as 4-year likelihood of coronary event in high-risk populations Proprietary test: SOMAmer® Lab/Manufacturer: SomaLogic
0052U	Lipoprotein, blood, high resolution fractionation and quantitation of lipoproteins, including all five major lipoprotein classes and subclasses of HDL, LDL, and VLDL by vertical auto profile ultracentrifugation Proprietary test: VAP Cholesterol Test Lab/Manufacturer: VAP Diagnostics Laboratory, Inc
0308U	Cardiology (coronary artery disease [CAD]), analysis of 3 proteins (high sensitivity [hs] troponin, adiponectin, and kidney injury molecule-1 [KIM-1]), plasma, algorithm reported as a risk score for obstructive CAD

CPT	Code Description
	Proprietary test: HART CAD® Lab/Manufacturer: Atlas Genomics
0309U	Cardiology (cardiovascular disease), analysis of 4 proteins (NT-proBNP, osteopontin, tissue inhibitor of metalloproteinase-1 [TIMP-1], and kidney injury molecule-1 [KIM-1]), plasma, algorithm reported as a risk score for major adverse cardiac event Proprietary test: HART CVE® Lab/Manufacturer: Atlas Genomics
0377U	Cardiovascular disease, quantification of advanced serum or plasma lipoprotein profile, by nuclear magnetic resonance (NMR) spectrometry with report of a lipoprotein profile (including 23 variables) Proprietary test: Liposcale® Lab/Manufacturer: CIMA Sciences, LLC
0415U	Cardiovascular disease (acute coronary syndrome [ACS]), IL-16, FAS, FASLigand, HGF, CTACK, EOTAXIN, and MCP-3 by immunoassay combined with age, sex, family history, and personal history of diabetes, blood, algorithm reported as a 5-year (deleted risk) score for ACS Proprietary test: SmartHealth Vascular Dx™ Lab/Manufacturer: Morningstar Laboratories, LLC

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

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Revision History

Revision Date	Summary of Changes
09/04/2024	Reviewed and Updated: Updated the background, guidelines and recommendations, and evidence-based scientific references. Literature review necessitated the following changes to coverage criteria: Removed "to 6" from CC1.a.i., now reads: "i) Every 4 years for individuals ages 18 to 79 years." Lipid panel screening is enforced in the least restrictive manner and as such, CC was edited for clarity on allowed testing frequency. Addition of "annually" to CC1.d.. Now reads: "d) Annually for individuals on a long-term drug therapy that requires lipid monitoring (e.g., Accutane, anti-psychotics)." Updated CPT table: 84512 mapped to policy but missing from policy document, now remedied

Celiac Disease Testing

Policy Number: AHS – G2043 – Celiac Disease Testing	Prior Policy Name and Number, as applicable:
Initial Presentation Date: 09/18/2015 Last Review Date: February 1, 2025	

Policy Description

Celiac disease is a hereditary, chronic autoimmune disorder triggered by the ingestion of gluten, a protein found in wheat, rye, and barley. When an individual with celiac disease ingests gluten, the body mounts an immune response that attacks the small intestine. These attacks lead to damage on the villi within the small intestine, inhibiting nutrient absorption (CDF, 2018).

Related Policies

Policy Number	Policy Title
AHS-G2121	Laboratory Testing for the Diagnosis of Inflammatory Bowel Disease
AHS-G2155	General Inflammation Testing

Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in in the "Applicable State and Federal Regulations" section of this policy document.

1) For individuals who have been diagnosed with celiac disease and who are IgA sufficient, serologic testing with IgA anti-tissue transglutaminase (TTG) **MEETS COVERAGE CRITERIA** at the following intervals:

   a) At the first follow-up visit 3-6 months after diagnosis.

b) Every 6 months until normalization of anti-TTG levels has occurred.

c) Every 12-24 months thereafter.

2) For individuals who have been diagnosed with celiac disease and who are IgA deficient, testing for IgG endomysial antibodies, IgG deamidated gliadin peptide, or IgG TTG **MEETS COVERAGE CRITERIA** at the following intervals:

   a) At the first follow-up visit 3-6 months after diagnosis.

   b) Every 6 months until normalization of IgG levels has occurred.

   c) Every 12-24 months thereafter.

3) For individuals with signs and symptoms of celiac disease (see Note 1), serologic testing with the IgA anti-TTG **and** the total IgA test for the diagnosis of celiac disease **MEETS COVERAGE CRITERIA**.

4) For individuals at risk for celiac disease (see Note 1), when IgA anti-TTG is negative or weakly positive, testing for IgA endomysial antibodies **MEETS COVERAGE CRITERIA**.

5) For individuals with clinical suspicion of celiac disease (see Note 1) with an IgA deficiency, testing for IgG endomysial antibodies, IgG deamidated gliadin peptide, **or** IgG TTG **MEETS COVERAGE CRITERIA**.

6) Testing for IgA and IgG antibodies to deamidated gliadin peptides **MEETS COVERAGE CRITERIA** in **any** of the following situations**:**

   a) For individuals under 2 years of age with a clinical suspicion of celiac disease (see Note 1).

   b) For individuals over 2 years of age as a substitute for anti-TTG testing.

7) Genetic testing for HLA DQ2 and DQ8 **MEETS COVERAGE CRITERIA** in **any** of the following situations:

   a) For symptomatic individuals for whom other testing is undiagnostic.

   b) For symptomatic individuals with positive serology tests who are unable to undergo a biopsy evaluation.

8) For confirmation of celiac disease in individuals at high risk for celiac disease, regardless of the result of celiac disease serology testing, pathological examination of tissue obtained from a biopsy of the small intestine **MEETS COVERAGE CRITERIA**.

9) Rapid antigen point-of-care testing for anti-TTG **DOES NOT MEET COVERAGE CRITERIA**.

10) Panel testing, multiplex testing, or multi-analyte testing (for more than two analytes) for the diagnosis or evaluation of celiac disease **DOES NOT MEET COVERAGE CRITERIA**.

11) For asymptomatic individuals not at an increased risk for developing celiac disease (see Note 1), testing for celiac disease **DOES NOT MEET COVERAGE CRITERIA.**

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

12) For the diagnosis of celiac disease, testing for anti-reticulin antibodies **DOES NOT MEET COVERAGE CRITERIA**.

13) For the evaluation of celiac disease, testing of stool or saliva samples **DOES NOT MEET COVERAGE CRITERIA**.

14) Serologic testing using an HLA-DQ-gluten tetramer-based assay, including flow cytometry-based HLA-DQ-gluten tetramer assays, **DOES NOT MEET COVERAGE CRITERIA**.

NOTES:

Note 1: Signs and symptoms of celiac disease may include, but are not limited to, the following: unexplained chronic or intermittent diarrhea; unexplained weight loss; unexplained chronic or intermittent abdominal pain or bloating; recurrent nausea or vomiting; unexplained iron deficiency anemia; unexplained vitamin B12 or folate deficiency; unexplained liver transaminase elevations; autoimmune hepatitis; dermatitis herpetiformis; type 1 diabetes; intestinal blockages; unexplained subfertility or miscarriage; unexplained osteoporosis, osteomalacia, or low bone density; and/or primary biliary cirrhosis. Individuals with Down syndrome, Turner syndrome, or Williams-Beuren syndrome are also at high risk for celiac disease. Additionally, in pediatric patients, fatty stools, delayed puberty, amenorrhea, failure to thrive, stunted growth, and/or short stature may also be associated with celiac disease (Husby et al., 2020; NICE, 2022; NIDDK, 2016).

Table of terminology

Term	Definition
AAFP	American Academy of Family Physicians
ACG	American College of Gastroenterology
AGA	American Gastroenterological Association
ANG	Anti-native gliadin antibodies
BSG	British Society of Gastroenterology
BSPGHAN	British Society of Pediatrics Gastroenterology, Hepatology, and Nutrition
CD	Celiac disease
CMS	Centers for Medicare and Medicaid Services
DGP	Deamidated gliadin peptides
DTC	Direct to consumer
ELISA	Enzyme-linked immunosorbent assay
EMA	Endomysial antibodies
ESP-GHAN	European Society for Pediatric Gastroenterology, Hepatology, and Nutrition
ESsCD	European Society for the Study of Coeliac Disease
GFD	Gluten-free diet
HLA	Human leukocyte antigen
HLA-DQA1	*Major histocompatibility complex, class II, DQ alpha 1 gene*
HLA-DQB1	*Major histocompatibility complex, class II, DQ beta 1 gene*

IgA	Immunoglobulin A
LDTs	Laboratory-developed tests
NASPGHAN	North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition
SSCD	Society for the Study of Celiac Disease
NCGS	Non celiac gluten sensitivity
NICE	National Institute for Health and Care Excellence
NIDDK	National Institute of Diabetes and Digestive and Kidney Diseases
sIgE	Specific immunoglobulin-E
SSOP	Sequence-specific oligonucleotide probe
TIDM	Type I diabetes mellitus
TTG	Tissue transglutaminase
ULN	Upper limit of normal
USPSTF	United States Preventative Services Task Force
WA	Wheat allergy
WGO	World Gastroenterology Organization
ZED1227	Oral transglutaminase 2 inhibitor

Scientific Background

Celiac disease (CD) is an autoimmune disease which occurs due to the body's unfavorable response after the ingestion of gluten. In particular, the body's immune system attacks the small intestine, leading to damage and inhibiting nutrient absorption (CDF, 2018). The clinical presentation of CD is varied and age dependent. In children, failure to thrive, malnutrition, diarrhea, abdominal pain, and distension are common. In adults, abdominal pain, diarrhea or constipation, bloating, and excessive gas are frequent symptoms. Other gastrointestinal symptoms include unexpected weight loss and distension (Kelly, 2023). A high prevalence of CD cases are often found in first degree relatives of CD patients, highlighting genetic aspects of the disease (Nellikkal et al., 2019). Currently, the only treatment for CD is to maintain a gluten-free diet to ameliorate symptoms and improve the quality of life (Caio et al., 2019).

The National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK, 2020) provides the following statistics for CD:

- About 2 million Americans have CD
- About 1% of people worldwide have CD
- The majority of people are undiagnosed
- CD can affect all races, but is present at a higher rate in Caucasians
- CD can affect both genders
- CD is more common among people with chromosomal disorders like Down syndrome, Turner syndrome, and Williams syndrome
- Patients with CD are at risk for Addison's disease, Hashimoto's disease, selective IgA deficiency, primary biliary cholangitis, and type 1 diabetes

CD has a strong genetic component. The two primary genetic factors for CD susceptibility are the human leukocyte antigen (*HLA*)-*DQ2* and *DQ8* alleles (Brown et al., 2019). These genes highlight the role of T cells and the immune response in CD (Tye-Din et al., 2018). Approximately 90-95% of CD patients

have the HLA-DQ2 protein encoded by the *HLA-DQA1*05* and *DQB1*02* alleles. The remaining CD patients have mutations in the HLA-DQ8 protein encoded by the *HLA-DQA1*03* and *DQB1*03:02* alleles. Stankovic et al. (2014) noted that the absence of susceptible *HLA-DQ* genotypes makes CD "very unlikely, close to 100%." However, the use of genotyping in diagnosing CD is not without controversy. Paul et al. (2017) report that 25-40% of white Caucasians are positive for the HLA-DQ2/DQ8 haplotype but that only 0.1-1% of the population will develop CD. They also note that the European guidelines released in 2012 recommend genotyping for HLA-DQ2/DQ8 in children with very high anti-TTG titers, but the authors recommend that "HLA-DQ2/DQ8 testing must not be done to 'screen' or 'diagnose' children" with CD (Paul et al., 2017).

Antibodies for the assessment of CD generally fall into one of two categories: autoantibodies (tTG-IgA, anti-endomysial antibody [EMA-IgA]) or antibodies targeting gliadin (DGP-IgA or IgG, antigliadin antibody (AGA)-IgA or IgG). Endomysial antibodies bind to tissue transglutaminase and produce a characteristic staining pattern. Similarly, anti-endomysial antibodies bind to tTG-2, another tissue transglutaminase. The other category of celiac antibodies involve gliadin, which is a component of gluten. Traditional antigliadin antibody tests (AGA-IgA, AGA-IgG) yielded a false positive rate of up to 20%, so they have been replaced with a deamidated gliadin peptide (DGP) (Kelly, 2023).

Genetic testing for HLA DQ2 and DQ8 may also be used to confirm a CD diagnosis. Serologic and histologic HLA-DQ testing requires the patient to be on a gluten-containing diet, which can be a disadvantage to testing. Recently, testing methods for HLA-DQ-gluten tetramer-based assays using flow-cytometry have been developed; these tests can accurately determine whether the patient is on a gluten-containing or gluten-free diet. The assay has a reported 97% sensitivity and 95% specificity for patients on a gluten-free diet as compared to controls (patients without CD) (Sarna et al., 2018). The authors conclude, "This test would allow individuals with suspected celiac disease to avoid gluten challenge and duodenal biopsy, but requires validation in a larger study" (Sarna et al., 2018).

Point of care tests, such as the Simtomax®, have been developed, which detects IgA and IgG antibodies against deamidated gliadin peptides (DGP) and provides a response in just ten minutes (Arenda, 2020). There are also direct-to-consumer (DTC) tests for CD. The FDA-approved 23andme panel includes CD. This test detects a single nucleotide polymorphism in HLA-DQA1 (FDA, 2017).

Clinical Utility and Validity

Olen et al. (2012) evaluated the diagnostic performance and actual costs in clinical practice of immunoglobulin (Ig)G/IgA DGP (deamidated gliadin peptide antibodies) as a complement to IgA-TTG for the diagnosis of pediatric CD. The authors identified 278 children with CD that received a duodenal biopsy. Sensitivity and specificity for tTG were 94% and 86% respectively, but corresponding values for DGP were 91% and 26%. Positive predictive values were 88% for tTG and 51% for DGP. The authors concluded that for diagnosing CD, tTG is superior to DGP, even in children younger than 2 years. Further, combining tTG and DGP does not provide a better trade-off between number of missed cases of CD, number of unnecessary duodenal biopsies, and cost than utilizing tTG alone (Olen et al., 2012).

Sakly et al. (2012) evaluated the usefulness of anti-DGP antibodies (a-DGP) in the diagnosis of CD. The study included 103 untreated CD patients of all ages and 36 CD patients under a gluten-free diet. The specificity of a-DGP was 93.6% for IgG and 92% for IgA as compared to the 100% for each by anti-endomysium antibodies (AEA) and tTG. The authors concluded that the findings of this study showed

"that a-DGP increases neither the sensitivity nor the specificity of AEA and AtTG [anti-tissue transglutaminase antibodies]" (Sakly et al., 2012).

Bufler et al. (2015) evaluated the diagnostic performance of three serological tests for CD. A total of 91 children with CD contributed 411 sera samples and were compared to 98 healthy controls. Transglutaminase type 2(TG2)-IgA, deamidated gliadin peptide (DGP)-IgG, and DGP-IgA were measured. The sensitivity for diagnosis was high for TG2-IgA and DGP-IgG (>90%) but lower for DGP-IgA. Specificity was >97% for all three. Non-adherence to a gluten-free diet was best indicated by positive TG2-IgA. The authors concluded that "combined testing for TG2-IgA and DGP-IgG does not increase the detection rate of CD in IgA competent children compared to TG2-IgA only" (Bufler et al., 2015).

Silvester et al. (2017) performed a meta-analysis to evaluate the "sensitivity and specificity of tTG IgA and EMA [endomysial antibody] IgA assays in identifying patients with celiac disease who have persistent villous atrophy despite a gluten-free diet (GFD)." The authors identified 26 studies for inclusion. The assays were found to have high specificity for identifying patients with persistent villous atrophy (0.83 for tTG IgA, 0.91 for EMA IgA, but with low sensitivity (0.50 for tTG IgA, 0.45 for EMA IgA). No significant difference was seen between pediatric and adult patients. The authors concluded that "we need more-accurate non-invasive markers of mucosal damage in children and adults with celiac disease who are following a GFD" (Silvester et al., 2017).

A report by Selleski et al. (2018) shows that only some of the *DQ2/DQ8* alleles were significantly different between pediatric CD patients and pediatric non-CD patients. A total of 97% of the CD patients were positive for at least either *DQ2* or *DQ8*; however, 29.9% of the non-CD patients were also positive for *DQ2*. In fact, "No significant association was found between *DQ2.2* variant and celiac disease in the studied population (Selleski et al., 2018)." Previously, high regard had been given to *DQ2.2* variant as being a predisposing variant for CD (Mubarak et al., 2013). Finally, a rapid nucleic acid amplification test using multiplex ligation-dependent probe amplification (MLPA) to detect HLA-DQ2.2, HLA-DQ2.5, and HLA-DQ8 has been developed with a reported 100% specificity for those particular genotypes (Vijzelaar et al., 2016), but this test has not been FDA-approved for use in the United States.

Bajor et al. (2019) performed a meta-analysis focusing on the association between the HLA-DQB1*02 gene doses and the characteristics of CD. The authors identified 24 studies for inclusion in the review and observed that homozygosity of the *DQB1*02* allele led to more frequent classical CD (odds ratio [OR] 1.758). The gene dosing effect was more prominent in children (OR: 2.082). Atrophic histology (Marsh grade 3) was more prevalent with a double dose compared to a zero dose (OR: 2.626). No gene dosing effect was seen with diarrhea, age at diagnosis, severity of villous atrophy, or type 1 diabetes. The authors concluded that "A double dose of *HLA-DQB1*02* gene seems to predispose patients to developing classical CD and villous atrophy. Risk stratification by *HLA-DQB1*02* gene dose requires further clarification due to the limited available evidence" (Bajor et al., 2019).

Tangermann et al. (2019) completed a prospective study which included 1055 patients all tested for CD with the Simtomax point of care test. The Simtomax detects IgA and IgG antibodies against deamidated gliadin peptides (DGP). All results were compared to the gold standard: histologic analysis of duodenal biopsies. Of all patients who participated in this study, the overall CD prevalence was identified at 4.1%; the Simtomax identified CD with a 79% sensitivity, 94% specificity, 37% positive predictive value, and 99% negative predictive value (Tangermann et al., 2019). When the adult (n=888) and pediatric (n=167) patients were analyzed separately, the Simtomax was found to identify CD with 100% sensitivity and 95% specificity in adults, and 72% sensitivity in children; the authors note that the Simtomax test detected CD with a lower sensitivity than expected (Tangermann et al., 2019).

Profaizer et al. (2020) conducted a study to "evaluate the feasibility of using NGS-based [next-generation sequencing] HLA-B and DQ genotyping for clinical HLA disease association testing and provide direct comparison with the currently used clinical tests, including SSOP [sequence-specific oligonucleotide probe] genotyping, and real-time PCR [polymerase chain reaction] with melting chain analysis." The researchers focused on HLA alleles related to celiac disease, ankylosing spondylitis, abacavir hypersensitivity, carbamazepine hypersensitivity, and allopurinol hypersensitivity. With regards to CD and from 24 samples tested, there was a discrepancy with the *DQB1*03:40* allele with SSOP, real-time PCR, and NGS, but overall, with the different *HLA*-correlations the data has shown "HLA typing by NGS is superior to the existing clinical methods for identifying HLA alleles associated with disease or drug hypersensitivity and offers a viable approach for high volume clinical diagnostic laboratories," continuing to demonstrate the clinical utility of NGS and HLA-testing for CD (Profaizer et al., 2020).

Gould et al. (2021) evaluated CD serologic testing in asymptomatic patients with type 1 diabetes using immunoglobulin A anti-tissue transglutaminase, as there is an increased risk of type 1 diabetes among CD and vice versa. From screening 2,353 patients, the assay with IgA anti-tissue transglutaminase had a positive predictive value of 85.9% when referenced upper limit of normal and had a sensitivity and specificity of 100% and 38%, respectively. This study indicated the need for thresholds for diagnostic evaluation to be population-specific (i.e. to type 1 diabetics), and not taken from the overall population due to the increased risk (Gould et al., 2021).

Schuppan et al. (2021) assessed the efficacy and safety of a 6-weel treatment with ZED1227, a selective oral transglutaminase 2 inhibitor, at three dose levels compared with placebo, in adults with well-controlled celiac disease who underwent a daily gluten challenge. Their primary endpoint was the attenuation of gluten-induced mucosal damage, measured by the ratio of villus height to crypt death. For this study, 41 patients were assigned to the 10-mg ZED1227 group, 41 patients were assigned to the 100-mg group, and 40 patients were assigned to the placebo group. Each had adequate duodenal-biopsy samples for the assessment of the overall endpoint. "The estimated difference from placebo in the change in the mean ratio of villus height to crypt depth from baseline to week 6 was 0.44 (95% confidence interval [CI], 0.15 to 0.73) in the 10-mg group (P = 0.001), 0.49 (95% CI, 0.20 to 0.77) in the 50-mg group (P<0.001), and 0.48 (95% CI, 0.20 to 0.77) in the 100-mg group (P<0.001). The estimated differences from placebo in the change in intraepithelial lymphocyte density were -2.7 cells per 100 epithelial cells (95% CI, -7.6 to 2.2) in the 10-mg group, -4.2 cells per 100 epithelial cells (95% CI, -8.9 to 0.6) in the 50-mg group, and -9.6 cells per 100 epithelial cells (95% CI, -14.4 to -4.8) in the 100-mg group." The authors concluded that treatment with ZED1227 attenuated gluten-induced duodenal mucosal damage in patients with celiac disease. (Schuppan et al., 2021).

Guidelines and Recommendations

American College of Gastroenterology (ACG)

The ACG recommends testing for CD in the following scenarios (Rubio-Tapia et al., 2013):

1. "Patients with symptoms, signs, or laboratory evidence suggestive of malabsorption, such as chronic diarrhea with weight loss, steatorrhea, postprandial abdominal pain and bloating, should be tested for CD. (Strong recommendation, high level of evidence)"
2. "Patients with symptoms, signs, or laboratory evidence for which CD is a treatable cause should be considered for testing for CD. (Strong recommendation, moderate level of evidence)"

3. "Patients with a first-degree family member who has a confirmed diagnosis of CD should be tested if they show possible signs or symptoms or laboratory evidence of CD. (Strong recommendation, high level of evidence)"
4. "Patients with type I diabetes mellitus should be tested for CD if there are any digestive symptoms, or signs, or laboratory evidence suggestive of celiac disease. (Strong recommendation, high level of evidence)"
5. "Celiac disease should be sought among the explanations for elevated serum aminotransferase levels when no other etiology is found, (Strong recommendation, high level of evidence)"
6. "Consider testing of asymptomatic relatives with a first-degree family member who has a confirmed diagnosis of CD (Conditional recommendation, high level of evidence)"

The ACG guidelines indicate that "Immunoglobulin A (IgA) anti-tissue transglutaminase (TTG) antibody is the preferred single test for detection of CD in individuals over the age of 2 years." Also, if there is "a high probability of CD wherein the possibility of IgA deficiency is considered, total IgA should be measured." Additionally, "an alternative approach is to include both IgA and IgG-based testing, such as IgG-deamidated gliadin peptides (DGPs), in these high-probability patients." In those patients with low or deficient IgA, the ACG recommends "IgG-based testing (IgG DGPs and IgG TTG)." The guidelines also indicate that all serological testing should be done while the individual is on a gluten-containing diet.

Intestinal biopsy is recommended by the ACG for individuals with positive serology testing and for those with a clinical presentation consistent with CD, "even if the serologies are negative."

Although antibodies directed against native gliadin are not recommended for the primary detection of CD," the ACG notes that "when screening children younger than 2 years of age for CD, the IgA TTG test should be combined with DGP (IgA and IgG)."

With regard to HLA-DQ2 / DQ8 genotype testing, the ACG recommends that it "should not be used routinely in the initial diagnosis of CD" but rather "should be used to effectively rule out the disease in selected clinical situations" such as, "equivocal small-bowel histological finding (Marsh I-II) in seronegative patients; evaluation of patients on a GFD in whom no testing for CD was done before GFD; patients with discrepant celiac-specific serology and histology; patients with suspicion of refractory CD where the original diagnosis of celiac remains in question; or patients with Down's syndrome… Because HLA-DQ2 is present in approximately 25%–30% of the white population, testing for CD with either HLA-DQ type is not useful because the PPV is only about 12%." Concerning HLA typing, "HLA typing, and histological response may help to rule out or confirm the diagnosis of CD in patients with sero-negative CD."

The ACG does not recommend stool or salivary testing, indicating that are not validated for use in the diagnosis of CD.

The ACG advocates monitoring of adherence to a gluten-free diet, based on "a combination of history and serology." Additionally, "upper endoscopy with intestinal biopsies is recommended for monitoring in cases with lack of clinical response or relapse of symptoms despite a GFD."

Celiac Disease Diagnostic Testing Algorithm (Rubio-Tapia et al., 2013)

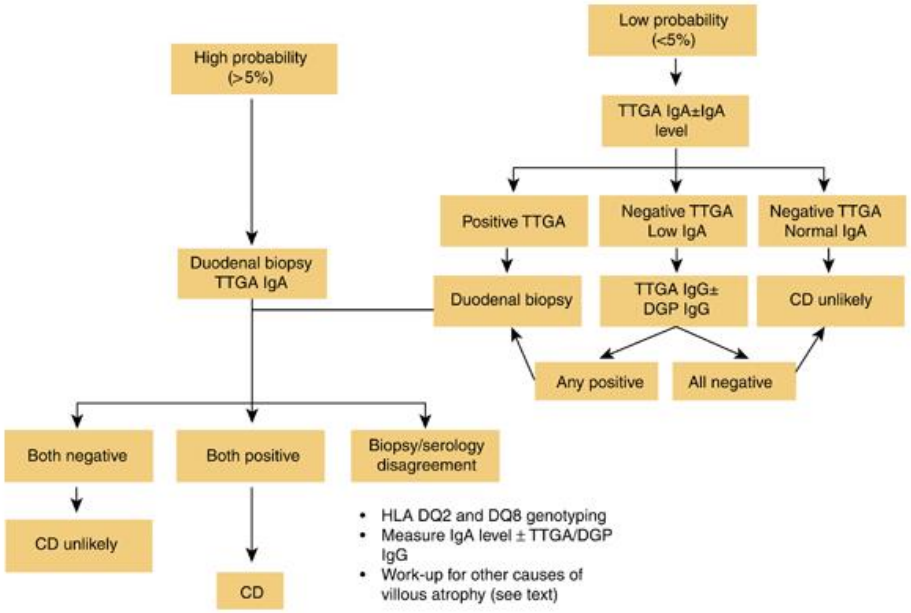


Figure 1. Celiac disease (CD) diagnostic testing algorithm. DGP, deamidated gliadin peptide; HLA, human leukocyte antigen; Ig, immunoglobulin; TTGA, tissue transglutaminase antibody

A 2023 update from ACG focused on the diagnosis and management of celiac disease expands upon their previous guidelines:

"1A. We [ACG] recommend EGD with multiple duodenal biopsies for confirmation of diagnosis in both children and adults with suspicion of CD (strong recommendation, moderate quality of evidence; dissent 1).

1B. We suggest a combination of high-level TTG IgA (>10× upper limit of normal) with a positive endomysial antibody (EMA) in a second blood sample as reliable tests for diagnosis of CD in children. In symptomatic adults unwilling or unable to undergo upper GI endoscopy, the same criteria may be considered after the fact, as a diagnosis of likely CD (conditional recommendation, moderate quality of evidence; dissent 0)."

ACG explains the above recommendations by citing key concepts:

"1. Multiple biopsies of the duodenum (1 or 2 from bulb and 4 from distal duodenum) are necessary for diagnosis of CD.

2. EGD and duodenal biopsies can also be useful for the differential diagnosis of other malabsorptive disorders or enteropathies.

3. Lymphocytic duodenosis (≥25 intraepithelial lymphocytes per 100 epithelial cells) in the absence of villous atrophy is not specific for CD, and other causes should be considered" (Rubio-Tapia et al., 2023).

Moreover, in the case of screening procedures, ACG states that

"7A. We recommend case finding to increase detection of CD in clinical practice (strong recommendation, low quality of evidence; dissent 0).

7B. We recommend against mass screening for CD in the community (strong recommendation, low quality of evidence; dissent 0)", on the basis that

"1. Patients with symptoms, signs, or laboratory evidence suggestive of malabsorption, such as chronic diarrhea with weight loss, steatorrhea, abdominal pain, and bloating, should be tested for CD.

2. Patients with symptoms, signs, or laboratory evidence for which CD is a treatable cause should be considered for testing for CD.

3. Patients with a first-degree family member who has a confirmed diagnosis of CD should be tested whether they show possible signs or symptoms or laboratory evidence of CD.

4. Consider testing of asymptomatic relatives with a first-degree family member who has a confirmed diagnosis of CD" (Rubio-Tapia et al., 2023).

Although they note that there is controversy surrounding the best serology approach for children younger than 2 years, ACG

"8A. We recommend the immunoglobulin IgA anti-TTG antibody (TTG-IgA) as the preferred single test for the detection of CD in children younger than 2 years who are not IgA-deficient (strong recommendation, moderate quality of evidence; dissent 0).

8B. We recommend that testing for CD in children with IgA deficiency be performed using IgG-based antibodies (DGP-IgG or TTG-IgG) (strong recommendation; moderate quality of evidence; dissent 0)" because

"1. TTG-IgA and EMA-IgA are reported to be less accurate in children younger than 2 years.

2. Current guidelines recommend that testing for CD in children younger than 2 years include both TTG-IgA and DGP-IgG" (Rubio-Tapia et al., 2023).

American Gastroenterological Association (AGA)

Relative to ongoing monitoring of individuals with celiac disease, the AGA recommends periodic serologic testing.

The AGA published an update on CD testing in 2019. Their new "best practice advice" is as follows:

- "Best Practice Advice 1: Serology is a crucial component of the detection and diagnosis of CD, particularly tissue transglutaminase–immunoglobulin A (TG2-IgA), IgA testing, and less frequently, endomysial IgA testing."
- "Best Practice Advice 2: Thorough histological analysis of duodenal biopsies with Marsh classification, counting of lymphocytes per high-power field, and morphometry is important for diagnosis as well as for differential diagnosis."
- "Best Practice Advice 2a: TG2-IgA, at high levels (> ×10 upper normal limit) is a reliable and accurate test for diagnosing active CD. When such a strongly positive TG2-IgA is combined with a positive endomysial antibody in a second blood sample, the positive predictive value for CD is

virtually 100%. In adults, esophagogastroduodenoscopy (EGD) and duodenal biopsies may then be performed for purposes of differential diagnosis."

- "Best Practice Advice 3: IgA deficiency is an infrequent but important explanation for why patients with CD may be negative on IgA isotype testing despite strong suspicion. Measuring total IgA levels, IgG deamidated gliadin antibody tests, and TG2-IgG testing in that circumstance is recommended."
- "Best Practice Advice 4: IgG isotype testing for TG2 antibody is not specific in the absence of IgA deficiency."
- "Best Practice Advice 5: In patients found to have CD first by intestinal biopsies, celiac-specific serology should be undertaken as a confirmatory test before initiation of a gluten-free diet (GFD)."
- "Best Practice Advice 6: In patients in whom CD is strongly suspected in the face of negative biopsies, TG2-IgA should still be performed and, if positive, repeat biopsies might be considered either at that time or sometime in the future."
- "Best Practice Advice 7: Reduction or avoidance of gluten before diagnostic testing is discouraged, as it may reduce the sensitivity of both serology and biopsy testing."
- "Best Practice Advice 8: When patients have already started on a GFD before diagnosis, we suggest that the patient go back on a normal diet with 3 slices of wheat bread daily preferably for 1 to 3 months before repeat determination of TG2-IgA."
- "Best Practice Advice 9: Determination of HLA-DQ2/DQ8 has a limited role in the diagnosis of CD. Its value is largely related to its negative predictive value to rule out CD in patients who are seronegative in the face of histologic changes, in patients who did not have serologic confirmation at the time of diagnosis, and in those patients with a historic diagnosis of CD; especially as very young children before the introduction of celiac-specific serology" (Husby et al., 2019).

The AGA's best advice statements for evaluating refractory celiac disease is recorded below.

Best Practice Advice 1

In patients believed to have celiac disease who have persistent or recurrent symptoms or signs, the initial diagnosis of celiac disease should be confirmed by review of prior diagnostic testing, including serologies, endoscopies, and histologic findings.

Best Practice Advice 2

In patients with confirmed celiac disease with persistent or recurrent symptoms or signs (nonresponsive celiac disease), ongoing gluten ingestion should be excluded as a cause of these symptoms with serologic testing, dietitian review, and detection of immunogenic peptides in stool or urine. Esophagogastroduodenoscopy with small bowel biopsies should be performed to look for villous atrophy. If villous atrophy persists or the initial diagnosis of celiac disease was not confirmed, consider other causes of villous atrophy, including common variable immunodeficiency, autoimmune enteropathy, tropical sprue, and medication-induced enteropathy.

Best Practice Advice 3

For patients with nonresponsive celiac disease, after exclusion of gluten ingestion, perform a systematic evaluation for other potential causes of symptoms, including functional bowel disorders, microscopic

colitis, pancreatic insufficiency, inflammatory bowel disease, lactose or fructose intolerance, and small intestinal bacterial overgrowth.

Best Practice Advice 4

Use flow cytometry, immunohistochemistry, and T-cell receptor rearrangement studies to distinguish between subtypes of refractory celiac disease and to exclude enteropathy-associated T-cell lymphoma. Type 1 refractory celiac disease is characterized by a normal intraepithelial lymphocyte population and type 2 is defined by the presence of an aberrant, clonal intraepithelial lymphocyte population. Consultation with an expert hematopathologist is necessary to interpret these studies.

Best Practice Advice 5

Perform small bowel imaging with capsule endoscopy and computed tomography or magnetic resonance enterography to exclude enteropathy-associated T-cell lymphoma and ulcerative jejunoileitis at initial diagnosis of type 2 refractory celiac disease.

Best Practice Advice 6

Complete a detailed nutritional assessment with investigation of micronutrient and macronutrient deficiencies in patients diagnosed with refractory celiac disease. Check albumin as an independent prognostic factor. (Green et al., 2022)

European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN)

Updated and expanded evidence-based guidelines for diagnosing CD were published in 2020 by the ESPGHAN. The following recommendations were included (Husby et al., 2020).

- "We recommend considering testing for CD in children and adolescents with symptoms, signs and conditions shown in Table 2"
    - Signs and symptoms in Table 2 include:
        - "Gastrointestinal: chronic or intermittent diarrhea, chronic constipation not responding to usual treatment, chronic abdominal pain, distended abdomen, recurrent nausea, recurrent vomiting
        - Extraintestinal symptoms: weight loss, failure to thrive, stunted growth/short stature, delayed puberty, amenorrhea, irritability, chronic fatigue, neuropathy, arthritis/arthralgia, chronic iron-deficiency anemia, decreased bone materialization (osteopenia/osteoporosis), repetitive fractures, recurrent aphthous stomatitis, dermatitis herpetiformis-type rash, dental enamel defects, abnormal liver biochemistry
        - Specific conditions: first degree relatives with CD, autoimmune conditions: TIDM, thyroid disease, liver disease, Down syndrome, Turner syndrome, Williams-Beuren syndrome, IgA deficiency"
- "HLA- typing does not add to the certainty of the diagnosis if the other criteria for CD diagnosis are fulfilled. Testing for HLA DQ2 and DQ8 may be useful in other circumstances. If no risk alleles are found, CD is unlikely. We recommend that HLA typing is not required in patients with positive TGA-IgA, if they qualify for CD diagnosis with biopsies or if they have high serum TGA-IgA (≥10× ULN) and EMA-IgA positivity. If a patient tests negative for HLA DQ2 and DQ8, the risk of CD is very low, while a positive result does not confirm the diagnosis"
- "Recent studies suggest that the no-biopsy approach to diagnose CD can be applied in asymptomatic children. In asymptomatic children, however, the PPV of high TGA-IgA ≥10× ULN

may be lower than in symptomatic children, which needs to be considered during the decision-making process. We give a conditional recommendation that, taking available evidence into account, CD can be diagnosed without duodenal biopsies in asymptomatic children, using the same criteria as in patients with symptoms. We recommend that the decision whether or not to perform diagnostic duodenal biopsies should be made during a shared decision-making process together with the parent(s) and, if appropriate, with the child"

- "The three specific coeliac antibodies (TGA-IgA, EMA-IgA, DGP-IgG) show different performance. TGA-IgA scored highest by a comparison of assay accuracy and is therefore regarded as the most appropriate primary test for CD in the diagnostic work up of children with suspected CD. We recommend that in subjects with normal serum IgA values for age, TGA-IgA should be used as the initial test regardless of age"
- "We recommend testing for total IgA and TGA-IgA as initial screening in children with suspected CD. In patients with low total IgA concentrations, an IgG-based test (DGP, EMA, or TGA) should be performed as a second step. Testing for EMA, DGP or AGA antibodies (IgG and IgA) as initial screening in clinical practice is not recommended"
- "We recommend that for CD diagnosis without biopsies, TGA-IgA serum concentration of at least 10× ULN should be obligatory. Only antibody tests with proper calibrator curve-based calculation, and having the 10× ULN value within their measurement range, should be used. We recommend against omitting biopsies in IgA-deficient cases with positive IgG-based serological tests"
- "We recommend that in children with TGA ≥10X ULN, and parents/patient agreement to the no-biopsy approach, the CD diagnosis should be confirmed by a positive EMA-IgA test in a second blood sample"
- "At least 4 biopsies from the distal duodenum and at least 1 from the duodenal bulb should be taken for histology assessment during a gluten-containing diet. Reading of biopsies should be performed on optimally orientated biopsies. A villous to crypt ratio of <2 indicates mucosal lesions. In cases of discordant results between TGA-IgA results and histopathology, re-cutting of biopsies and/or second opinion from an experienced pathologist should be requested (Husby et al., 2020)"

A 2022 position paper on the management and follow-up of children and adolescents with celiac disease stated that

"3.1 The first follow-up visit should be scheduled 3–6 months after CD diagnosis, but with easy access to the celiac service if earlier advice is needed, and sooner review if there are concerns regarding how the family is coping with the diet, if there are ongoing issues with growth or persistent symptoms or a need to repeat bloodwork earlier. Subsequent visits should be every 6 months until normalization of TGA levels, and every 12–24 months thereafter."

"3.2. During follow-up patients should be evaluated for:

3.2.I. Gastrointestinal and extraintestinal signs and symptoms.

3.2.II. Anthropometric measurements and growth parameters.

3.2.III. IgA-TGA using the same assay as at diagnosis as a surrogate marker for improvement/healing of the small-bowel mucosa. IgG based tests and RIA based IgA-TGA measurements are not suitable for follow-up in IgA sufficient patients. IgA insufficient patients with CD should be followed with IgG based tests.

3.2.IV. A complete blood cell count, micronutritional status (e.g., hemoglobin, iron, vitamin B12, and vitamin D levels) and ALT measurements, should be performed after clinical evaluation at time of diagnosis. Any abnormality should be followed and deficiencies corrected until normalization. If abnormalities persist, additional diagnoses should be considered and appropriately investigated.

3.2.V. Screening for thyroid disease with TSH and thyroxine (and autoantibodies if indicated) may be considered during follow-up after clinical evaluation at the discretion of the clinician.

3.2.VI. Routine bone-density screening is not recommended.

3.2.VII. HBV antibody levels may be measured in previously immunized patients if this is considered important in the population. A booster dose should be given if inadequate levels are present" (Mearin et al., 2022).

A few specific issues of note that may come up during follow-up and management include

"6.1. **How to approach persistent high serum levels of antibodies against tissue-transglutaminase (TGA)?**

Lack of decreasing IgA-TGA levels after 6–12 months on a GFD or persisting positive IgA-TGA levels should be assessed by carefully reviewing dietary compliance and testing IgA-TGA using the same test from the same manufacturer.

6.2. **When is it necessary to (re)biopsy?**

Routine assessment of mucosal healing by small-bowel biopsies is not recommended in children with CD following a GFD. We recommend considering (re) biopsy only in selected CD cases; based on specific clinical grounds, for example, when doubts about the original diagnosis or suspicion of occurrence of an additional condition.

6.3. **Refractory celiac disease in children: does it exist?**

We recommend properly investigating other causes of an apparent "refractory CD" in children, including ongoing inadvertent ingestion of gluten and other possible concomitant enteropathies, such as Crohn's disease, autoimmune enteropathy, small-bowel bacterial overgrowth, cow's milk protein allergy and pancreatic insufficiency" (Mearin et al., 2022).

ESPGHAN suggests that "In cases of uncertain CD diagnosis, HLA typing should be performed before gluten-challenge to detect children in whom the occurrence of CD is unlikely." Moreover, they "recommend the same frequency and follow-up tests in children with CD and T1D as inchildren with isolated CD, with (additional) special attention to test for thyroid involvement and diabetic retinopathy" and that "developing the follow-up plan in conjunction with an endocrinologist/diabetologist and a dietitian, also considering the need for psychological and social support" (Mearin et al., 2022).

In 2012, ESPGHAN recommended that CD testing be considered for "children and adolescents with the otherwise unexplained symptoms and signs of chronic or intermittent diarrhoea, failure to thrive, weight loss, stunted growth, delayed puberty, amenorrhoea, iron-deficiency anaemia, nausea or vomiting, chronic abdominal pain, cramping or distension, chronic constipation, chronic fatigue, recurrent aphthous stomatitis (mouth ulcers), dermatitis herpetiformis–like rash, fracture with inadequate traumas/osteopenia/osteoporosis, and abnormal liver biochemistry." Testing should also be offered to

"asymptomatic children and adolescents with an increased risk for CD such as type 1 diabetes mellitus (T1DM), Down syndrome, autoimmune thyroid disease, Turner syndrome, Williams syndrome, selective immunoglobulin A (IgA) deficiency, autoimmune liver disease, and first-degree relatives with CD" (Husby et al., 2012).

ESPGHAN recommends that "the initial test be IgA class anti-TG2 from a blood sample. If total serum IgA is not known, then this also should be measured." If the individual has humoral IgA deficiency, "at least 1 additional test measuring IgG class CD-specific antibodies should be done (IgG anti-TG2, IgG anti-DGP or IgG EMA." They also note that "tests measuring antibodies against DGP may be used as additional tests in patients who are negative for other CD-specific antibodies but in whom clinical symptoms raise a strong suspicion of CD, especially if they are younger than 2 years," and "tests for the detection of IgG or IgA antibodies against native gliadin peptides (conventional gliadin antibody test) should not be used for CD diagnosis." They also indicate that "tests for the detection of antibodies of any type in faecal samples should not be used."

For individuals with "severe symptoms and a strong clinical suspicion of CD" and negative serology testing, "small intestinal biopsies and HLA-DQ testing are recommended."

Regarding the evaluation of asymptomatic children and adolescents with CD-associated conditions, ESPGHAN recommends HLA testing "should be offered as the first line test," due to its high negative predictive value. "If the patient is DQ8 and/or DQ2 positive, homozygous for only the b chains of the HLA-DQ2 complex (DQB1_0202), or HLA testing is not done, then an anti-TG2 IgA test and total IgA determination should be performed, but preferably not before the child is 2 years old. If antibodies are negative, then repeated testing for CD-specific antibodies is recommended" (Husby et al., 2012).

ESPGHAN also recommends that in asymptomatic individuals at increased genetic risk for CD "duodenal biopsies with the demonstration of an enteropathy should always be part of the CD diagnosis." As an initial step, "it is recommended that the more specific test for EMA be performed. If the EMA test is positive, then the child should be referred for duodenal biopsies. If the EMA test is negative, then repeated serological testing on a normal gluten-containing diet in 3 to 6 monthly intervals is recommended" (Husby et al., 2012). Testing of infants, as with all serologic testing for CD, should be done only when the individual is on a gluten-containing diet.

North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition (NASPGHAN)

NASPGHAN updated their recommendations in 2015 (published in 2016) for gluten-related disorders, including CD, wheat allergy (WA), and nonceliac gluten sensitivity (NCGS). Concerning who should be tested for gluten-related disorders, "Children with symptoms consistent with gluten-related disorders, or who have self-identified relief of symptoms when avoiding gluten, should undergo testing for CD and/or WA before the elimination of dietary gluten. CD should be an early consideration in those with typical gastrointestinal symptoms such as chronic diarrhea, abdominal pain, distension, and weight loss." The table below outlines their recommendations for considering CD testing:

TABLE 2. Indications to consider CD testing

Symptoms	Associated conditions
Abdominal pain	First-degree relatives of those with CD
Abdominal distension	Type 1 diabetes
Diarrhea	Autoimmune thyroid disease
Constipation	Autoimmune liver disease
Growth failure or deceleration	Trisomy 21
Weight loss	Williams syndrome
Arthralgia	Turner syndrome
Elevated hepatic transaminases	IgA deficiency
Iron deficiency anemia	Juvenile chronic arthritis
Unexplained osteopenia	
Dental enamel defects	
Recurrent aphthous stomatitis	
DH	

CD = celiac disease; DH = dermatitis herpetiformis; IgA = immunoglobulin A.

"Children belonging to groups known to be at increased risk for CD may initially have no symptoms, or very minor symptoms, despite having intestinal histologic changes that are characteristic for CD. Included in these groups are first-degree relatives of an index case, people with trisomy 21, Turner syndrome, Williams syndrome, and IgA deficiency, and those with other autoimmune conditions" (Hill et al., 2016).

For initial testing, they recommend the TTG-IgA antibody test due to its reliability and cost-effectiveness. They note that co-testing for serum IgA can be performed to "identify those who have selective IgA deficiency"; however, "use of a panel of antibodies instead of a single tTG-IgA test is not recommended. Although this approach may be associated with a marginal increase in the sensitivity of the test, it decreases the specificity and significantly increases the costs" (Hill et al., 2016). Testing for serum antibodies against gliadin is less sensitive, reliable, and specific as compared to TTG and EMA.

They do not recommend genetic testing for *HLA* variants as an initial diagnostic test or screening for CD since up to 40% of the general population contains one of the variant alleles. "Testing for HLA-DQ2/8 is best reserved for patients in whom there is a diagnostic dilemma, such as when there is a discrepancy between the serological and histologic findings or when a GFD [gluten-free diet] has been started before any testing" (Hill et al., 2016).

They do not recommend the use of rapid, point-of-care tests for TTG since these tests do not allow for the quantitative analysis of the antibody.

National Institute for Health and Care Excellence (NICE)

In 2022, NICE published guidance on diagnosing CD. These guidelines state that serological testing should be offered to "people with any of the following: persistent unexplained abdominal or gastrointestinal symptoms, faltering growth, prolonged fatigue, unexpected weight loss, severe or persistent mouth ulcers, unexplained iron, vitamin B12 or folate deficiency, type 1 diabetes, at diagnosis, autoimmune thyroid diseases, at diagnosis, irritable bowel syndrome (in adults), [and] first-degree relatives of people with celiac disease" (NICE, 2022).

"Any test is accurate only if a gluten-free containing diet is eaten during the diagnostic process **and** advise the person not to start a gluten-free diet until diagnosis is confirmed by a specialist, even if the results of a serological test are positive" (NICE, 2022).

Further, serological testing for CD could be considered in patients with "metabolic bone disorder (reduced bone mineral density or osteomalacia), unexplained neurological symptoms (particularly peripheral neuropathy or ataxia), unexplained subfertility or miscarriage, persistently raised liver enzymes with unknown cause, dental enamel defects, Down's syndrome, and Turner syndrome" (NICE, 2022).

Finally, regarding serological testing:

- "Test for total IgA and IgA tTG as the first choice
- Use IgA EMA if IgA tTG is weakly positive
- Consider using IgG EMA, IgG DGP or IgG tTG if IgA is deficient (IgA deficiency is defined as total IgA less than 0.07 g per litre) (NICE, 2022)."

In 2015, the National Institute for Health and Care Excellence (NICE) recommended CD serologic testing in symptomatic young people and adults with the following algorithm (NICE, 2015):

- First test for total serum IgA and TTG
- Next test for IgA endomysial antibodies (EMA) if TTG is inconclusive (i.e., weakly positive)
- "Consider using IgG EMA, IgG deamidated gliadin peptide (DGP) or IgG tTG if IgA is deficient"

For children with suspected CD, they recommend:

- First test for total serum IgA and TTG
- "Consider using IgG EMA, IgG DGP or IgG tTG if IgA is deficient"

NICE also recommends offer CD testing for people with any of the following:

- Autoimmune thyroid disease
- Persistent unexplained abdominal or gastrointestinal symptoms
- Irritable bowel syndrome
- Type 1 diabetes
- First-degree relatives (parents, siblings, or children) with coeliac disease
- Other symptoms indicative of possible CD, including faltering growth in children, prolonged fatigue, unexpected weight loss, severe or persistent mouth ulcers, unexplained dietary deficiencies

NICE also recommends considering CD testing for people with the following:

- Metabolic bone disorder
- Unexplained neurological symptoms
- Unexplained subfertility or recurrent miscarriage
- Down's syndrome or Turner's syndrome
- Dental enamel defects
- Persistent elevated hepatic enzymes of unknown etiology

They do note that "People who are following a normal diet (containing gluten) should be advised to eat gluten in more than 1 meal every day for at least 6 weeks before testing for coeliac disease" (NICE, 2016).

NICE indicates that HLA testing should not be done as part of the initial testing. Also, "Only consider using HLA DQ2 (DQ2.2 and DQ2.5)/DQ8 testing in the diagnosis of coeliac disease in specialist settings (for example, in children who are not having a biopsy, or in people who already have limited gluten ingestion and choose not to have a gluten challenge)" (NICE, 2015).

United States Preventive Services Task Force (USPSTF)

The United States Preventative Services Task Force (Bibbins-Domingo et al., 2017) recently published guidelines on the screening of asymptomatic populations for celiac disease and found that

"The USPSTF concludes that the current evidence is insufficient to assess the balance of benefits and harms of screening for celiac disease in asymptomatic persons. Evidence is lacking, and the balance of benefits and harms cannot be determined." However, it was noted that: "Persons at increased risk for celiac disease include those who have a positive family history (eg, a first- or second-degree relative), with an estimated prevalence of 5% to 20%, and persons with other autoimmune diseases (eg, type 1 diabetes mellitus, inflammatory luminal gastrointestinal disorders, Down syndrome, Turner syndrome, IgA deficiency, and IgA nephropathy). Several specialty societies recommend screening in these populations."

World Gastroenterology Organisation (WGO) Global Guidelines

The WGO published guidelines on CD testing in 2017. A cascade with "resource-sensitive" options is listed.

The "Gold Standard" lists the following items for diagnosis of CD:

- Celiac disease–specific antibodies: assessment and intestinal biopsy
- Anti-tTG IgA or anti-EMA IgA, and total IgA to exclude IgA deficiency
- In case of selective IgA deficiency, IgG-based tests should be used: anti-DGP, anti-tTG, or EMA (the latter 2 are highly sensitive, but with lower specificity)
- Symptomatic patients with a positive serological test or a titer just below the cut-off (borderline) should be referred for endoscopy with multiple duodenal biopsies to confirm or exclude the diagnosis of celiac disease. Pitfalls in histologic diagnosis are common, and findings are characteristic, but not specific
- Asymptomatic patients with a positive serological test should be retested after consuming a gluten-containing diet for 3 months, to confirm persistent seropositivity before referral for endoscopy

The following items are listed for management of CD:

- Follow-up monitoring, including antibody tests (anti-tTG IgA or DGP-IgG in case of IgA deficiency): after 3 to 6 months in the first year and once a year thereafter in stable patients responding to the gluten-free diet

The WGO also notes that although the presence of HLA risk alleles is "necessary" for celiac disease, it is insufficient for CD development. However, it does have a high negative predictive value, in that absence of those risk alleles excludes CD as a diagnosis.

The WGO notes two main groups of serological markers for untreated CD:

- Autoantibodies targeting the auto-antigen: EMA and anti-tTG antibodies
- Antibodies targeting the offending agent (gliadin): anti- bodies against synthetic deamidated gliadin peptides (anti-DGPs)

A summary of the characteristics of CD antibody tests is listed below:

TABLE 8. Sensitivity and Specificity and General Characteristics of Celiac Disease–specific Antibody Tests

Test	Methods	Sensitivity	Specificity	Comments
Autoantibodies against tissue transglutaminase (anti-tTG) IgA	Commercial ELISA, in-house ELISA, RIA, and other methods; cut-off given in arbitrary units, which differ from test to test	Very good	Very good	Autoantibody against auto-antigen in celiac disease. Considered by most guidelines as the first-line test for screening, but should be combined with a test based on total IgA or total IgG
Autoantibodies against tissue transglutaminase (anti-tTG) IgG	Commercial ELISA, in-house ELISA, RIA, and other methods, can be combined with an IgA-based test; cut-off given in arbitrary units, which differ from test to test	Very good in IgA-deficient patients Lower in IgA-competent patients	—	Should be used in case of IgA deficiency; combined IgA and IgG-based tests are available
Autoantibodies against endomysium (EMA) IgA	Immunofluorescence on tissue slides; commercially available, results are given in serum dilution with the first positive signal (1:2.5, 1:5, 1:10, etc.) or as positive/negative only	Less sensitive in comparison with anti-tTG IgA	Most specific test; should therefore be used as a confirmatory test if celiac disease diagnosis is made without duodenal biopsies	Requires an immunofluorescence microscope and experienced laboratory technician; more expensive
Autoantibodies against endomysium (EMA) IgG	See EMA IgA	Only used in patients with IgA deficiency	Confirmatory test in patients with IgA deficiency	See EMA IgA
Antibodies against deaminated gliadin peptide (anti-DGP) IgA	Various commercial or in-house ELISA or other methods; cut-off given in arbitrary units, which differ from test to test	Lower in comparison with anti-tTG IgA	Lower in comparison with anti-tTG IgA	May detect patients who are negative for tTG
Antibodies against deaminated gliadin peptide (anti-DGP) IgG	Various commercial or in-house ELISA or other methods; cut-off given in arbitrary units, which differ from test to test	Very good. Alternative test to anti-tTG IgG in patients with IgA deficiency	Slightly lower in comparison with anti-tTG IgA	A high rate of false-positive results has been reported in infants; may detect patients negative for tTG

Anti-DGP indicates antibodies to deaminated gliadin peptides; anti-tTG, antibodies to tissue transglutaminase-2; ELISA, enzyme-linked immunosorbent assay; EMA, endomysial antibodies; IgA, immunoglobulin A; IgG, immunoglobulin G; RIA, radioimmunoassay.

The WGO also lists several conditions associated with a higher risk of CD. Those conditions are as follows:

- Type 1 diabetes mellitus
- Autoimmune thyroid disease
- Autoimmune liver disease
- Down syndrome
- Turner syndrome
- Williams syndrome
- Selective IgA deficiency
- Unexplained elevated serum aminotransferase levels

The WGO also recommends that first-degree relatives of index (affected) patients to be screened for CD.

Finally, WGO recommends against use of urine, stool, or saliva measurements in clinical practice, as they have a "lower performance" than blood-based tests (Bai & Ciacci, 2017).

European Society for the Study of Coeliac Disease (ESsCD)

The ESsCD published guidelines on CD, including recommendations on serological and genetic testing. These recommendations are listed below:

- "Adult patients with symptoms, signs or laboratory evidence suggestive of malabsorption should be tested with serology for CD. (Strong recommendation, high level of evidence)"
- "Screening of asymptomatic first-degree family member of CD patient is recommended. If available, HLA-typing may be offered as the first-line test; if negative, no further work-up is needed. (Conditional recommendation, high level of evidence)"
- "CD should be excluded in patients with unexplained elevation of serum aminotransferase levels. (Strong recommendation, high level of evidence)"
- "T1DM should be screened regularly for CD. (Strong recommendation, high level of evidence)"
- "IgA-TG2 antibody is the preferred single test for detection of CD at any age. (Strong recommendation, high level of evidence)"
- "Total IgA level needs to be measured concurrently with serology testing to determine whether IgA levels are sufficient. (Strong recommendation, moderate level of evidence)"
- "In patients with selective total IgA-deficiency, IgG-based testing (IgG-DGPs or IgG-TG2) should be performed at diagnosis and follow-up. (Strong recommendation, moderate level of evidence)"
- "All diagnostic serologic testing should be done while patients on a gluten-containing diet. (Strong recommendation, high level of evidence)"
- "Antibodies directed against native gliadin (AGA) are not recommended for the primary detection of CD. (Strong recommendation, high level of evidence)"
- "Intestinal-permeability tests are neither sensitive nor specific and are not recommended for CD diagnosis. (Strong recommendation, moderate level of evidence)"
- "Serum I-FABP might be useful in identifying dietary non-adherence and unintentional gluten intake. (Strong recommendation, moderate level of evidence)"
- "A newly diagnosed adult CD patient should undergo testing to uncover deficiencies of essential micronutrient, e.g. iron, folic acid, vitamin D and vitamin B12. (Strong recommendation, moderate level of evidence)"
- "CD diagnosis may be made without duodenal biopsy in symptomatic children with high TG2 levels (>10 times ULN) and EMA in the presence of HLA-DQ2/8. The diagnosis is confirmed by an antibody decline and preferably a clinical response to a GFD". (Conditional recommendation, moderate level of evidence)

The ESsCD also lists recommendations for HLA-DQ2/8 typing, which are as follows:

- "A negative HLA test is helpful to exclude the possibility of CD. This is especially helpful in those already on a GFD before testing."
- "When diagnosis of CD is uncertain, e.g., negative serology, but histology suggestive of CD."
- "To distinguish siblings who can be reassured that it is unlikely that they will develop CD from those who need to be monitored. Furthermore, the data on the quality of life on a GFD in those patients detected by screening are conflicting, but there is a trend towards improvement. Also, the lack of understanding of the natural history of undiagnosed CD may justify screening asymptomatic persons."

- "In subjects with other autoimmune diseases and some genetic disorders who should be investigated for CD."
- "HLA-DQ2/DQ8 testing should not be used routinely in the initial diagnosis of CD. It is recommended that the results of such testing should be included along with a caution that patients at risk should be serologically tested for CD without changing their diet. (Strong recommendation, moderate level of evidence)" (Al-Toma et al., 2019).

British Society of Gastroenterology (BSG)

In 2014, the BSG published guidelines for the diagnosis and management of adult CD. The following guidelines were included:

- "Diagnosis of CD requires duodenal biopsy when the patient is on a gluten-containing diet and for the vast majority of adult patients also positive serology. (Grade B)
- Biopsy remains essential for the diagnosis of adult CD and cannot be replaced by serology. Follow-up should aim at strict adherence to a gluten-free diet. (Grade B)
- In individuals undergoing an upper endoscopy in whom laboratory tests or symptoms or endoscopic features suggest CD, duodenal biopsy should be considered. (Grade C)
- HLA typing should be used to rule out CD. A positive DQ2.5 or DQ8 can never confirm the diagnosis. (Grade B)
- HLA typing should be used in individuals who are self-treated on a GFD and never had appropriate testing for CD before changing their diet. (Grade B)
- HLA typing can be used to rule out CD, and minimise future testing, in high-risk individuals with CD, for example, first-degree relatives. (Grade B)
- The diagnosis of CD requires duodenal biopsy when the patient is on a gluten-containing diet and for the vast majority of adult patients also positive serology. (Grade B)
- Duodenal biopsy should be retained as the mainstay for the diagnosis of adult CD and cannot be replaced by serology. (Grade B)
- At endoscopy, if there is suspicion of CD, then at least four biopsy specimens should be obtained, including a duodenal bulb biopsy. (Grade C)
- In serologically negative patients showing signs of malabsorption (such as anaemia or diarrhoea) or a family history of CD, a duodenal biopsy should be considered. (Grade C)
- Follow-up biopsies may be considered in patients with CD, and are potentially helpful in identifying patients at increased risk of lymphoma. (Grade B)" (Ludvigsson et al., 2014)

British Society of Paediatric Gastroenterology, Hepatology, and Nutrition (BSPGHAN) and Coeliac UK

In 2013, the BSPGHAN and Coeliac UK published joint guidelines for the management of CD in children. These guidelines note that "Anti-tTG antibody positivity alone is insufficient for diagnosis. Therapeutic trials of GFD are NOT indicated if CD is suspected." Further, if the patient is symptomatic, IgA and IgA tTG should be checked first" (Murch et al., 2013).

- "If tTG negative and IgA normal, CD unlikely: If IgA low, then further testing (eg, IgG tTG and possible biopsy) is required.
- If tTG raised—but less than 10×upper limit of normal for assay: Duodenal biopsy is required. At endoscopy, take four biopsies from D2 or lower and 1–2 from duodenal bulb (as patchy changes may be present). Ensure adequate gluten intake prior to testing with advice from dietician if necessary

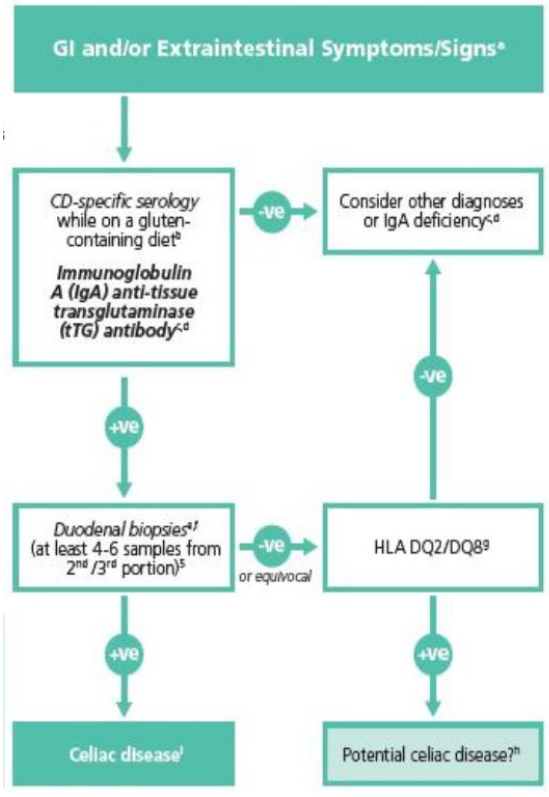
- If tTG raised—and greater than 10×upper limit of normal for assay: Take further blood sample to check IgA-EMA and determine HLA-DQ2/HLA-DQ8 typing. If EMA+ and patient either DQ2 or DQ8, the diagnosis is confirmed without the need for a duodenal biopsy. If EMA antibody testing is not locally available, a second strongly positive tTG antibody may be substituted and serum saved for later EMA testing" (Murch et al., 2013).

Society for the Study of Celiac Disease (SSCD)

In 2017, the NASSCD uploaded a guideline for celiac disease diagnosis in adults, along with a diagram detailing the algorithm for a positive celiac disease diagnosis with presenting GI and/or extraintestinal symptoms and signs. They state:

"Celiac disease (CD) may be suspected in

1. Symptomatic patients with
   - Gastrointestinal symptoms/signs: diarrhea, weight loss, gas/bloating, constipation (more commonly in children), hypertransaminasemia
   - Extraintestinal symptoms/signs: iron deficiency anemia, dermatitis herpetiformis, osteoporosis and neuropsychiatric conditions, such as neuropathy or ataxia
2. Patients with associated conditions
   - Type 1 diabetes mellitus
   - Autoimmune thyroiditis
   - Other autoimmune conditions
   - Down syndrome
3. First-degree family members of celiac patients[a]"



(+ve indicates positive, -ve indicates negative)

The NASSCD has also included the following comments with regards to its algorithm and suspicion of CD:

a. "Screening for celiac disease in high-risk asymptomatic populations is controversial due to unknown natural history and potential benefits.
b. If patient self-started a gluten-free diet, consider testing after challenge with >3g of gluten per day (equivalent to 1-2 slices of bread per day) for at least two weeks.
c. The addition of total IgA is useful to detect IgA deficiency.
d. An alternative approach is to include both IgA and IgG-based testing, such as IgG-deamidated gliadin peptides (DGPs).
e. There is scarce data using serology alone for diagnosis of CD. Combined use of biopsy and serologic analyses for diagnosis of celiac disease is recommended in adults.
f. Current guidelines recommend 1-2 biopsies from the bulb in addition to at least 4 biopsies from distal duodenum. However, the advantages, (increased sensitivity) and disadvantages (reduced specificity) of bulb biopsies are under scrutiny.
g. *HLA DQ2/DQ8* negative excludes CD in majority of cases.
h. Other reasons for discrepant serology and biopsies include reduced gluten in the diet, inadequate biopsy sampling, and lack of expert histopathology reporting.
i. Celiac disease diagnosis is confirmed after clinical and/or histology improvement after gluten-free diet" (SSCD, 2017).

American Academy of Family Physicians (AAFP)

In January 2014, the AAFP released a set of recommendations regarding the diagnosis and management of celiac disease. Based on "consensus, disease-oriented evidence, usual practice, expert opinion, or case series (Evidence Rating: C)," the AAFP stated the following:

- "Immunoglobulin A tissue transglutaminase should be used as the first-line test for serologic diagnosis of suspected celiac disease.
- Small bowel biopsy should be used to confirm the diagnosis of celiac disease in most patients" (Pelkowski & Viera, 2014).

A 2017 the AAFP adduces the USPSTF guidelines in their recommendation statement for the screening of celiac disease. The table the AAFP included from the USPSTF is shown below.

Recommendation

Table 1. Screening for Celiac Disease: Clinical Summary of the USPSTF Recommendation

Population	Asymptomatic adults, adolescents, and children
Recommendation	No recommendation. Grade: I (insufficient evidence)
Risk assessment	Persons at increased risk for celiac disease include those who have a positive family history (e.g., a first- or second-degree relative) and persons with other autoimmune diseases (e.g., type 1 diabetes mellitus, inflammatory luminal gastrointestinal disorders, Down syndrome, Turner syndrome, IgA deficiency, and IgA nephropathy).
Screening tests	Screening for celiac disease is typically not performed in average-risk persons. The standard method of diagnosing celiac disease is the tissue transglutaminase IgA test, followed by intestinal biopsy for histologic confirmation.
Treatment	Treatment of celiac disease is lifelong adherence to a gluten-free diet, which reverses disease manifestations in a majority of patients.
Balance of benefits and harms	The USPSTF concludes that the current evidence is insufficient to assess the balance of benefits and harms of screening for celiac disease in asymptomatic persons.

NOTE: *For a summary of the evidence systematically reviewed in making this recommendation, the full recommendation statement, and supporting documents, go to http://www.uspreventiveservicestaskforce.org/.*

USPSTF = U.S. Preventive Services Task Force.

(AAFP, 2017)

Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

The Quanta Lite Celiac Screen ELISA test for tissue transglutaminase/gliadin and the Quanta Lite Celiac DGP Screen by Inova Diagnostics, Inc. were approved by the FDA on 01/28/1999 and 12/13/2006, respectively. Quanta Plex Celiac IgA and IgG profiles by Inova Diagnostics, Inc. were approved on 03/14/2007 and 06/20/2007.

EliA Celikey IgG for use with the EliA Celikey IgG Immunoassay by Phadia US, Inc. was approved by the FDA on 12/26/2006.

The FIDIS Celiac on the FIDS Analyser and FIDIS CELIAC kit by Biomedical Diagnostics S.A. were approved by the FDA on 09/24/2004 and 03/29/2006, respectively.

The IMMULISA CELIAC ELISA testing systems for gliadin IgA/IgG and TTG IgA/IgG by IMMCO Diagnostics, Inc. were approved on 02/04/2010 and 03/10/2010. IMMCO's IMMULISA enhanced celiac fusion (TTG/DGP) IgA/IgG antibody ELISA system was approved on 10/25/2013.

Bio-Rad Laboratories' Bioplex 2200 Celiac IgA IgG kits were approved on 09/19/2013. The IgX Plex Celiac qualitative assay and Ig Plex Celiac DG panel by SQI diagnostics systems, Inc. were approved by the FDA on 06/02/2011 and 11/06/2014, respectively.

SQI Diagnostics received FDA clearance for the Ig plex Celiac DGP which detects IgA and IgG antibodies to deamidated gliadin peptide (DGP) and tissue transglutaminase (tTG) in human serum. This was approved by the FDA on Nov 06, 2014 (FDA, 2014).

Inova Diagnostics received FDA clearance on June 16, 2021, for the Aptiva Celiac Disease IgA Reagent, which is an "immunoassay utilizing particle-based multi-analyte technology for the semi-quantitative determination of anti-tissue transglutaminase IgA autoantibodies and anti-deamidated gliadin peptide IgA antibodies in human serum." It can be used to diagnose celiac disease and dermatitis herpetiformis (FDA, 2021).

No nucleic acid-based test solely for celiac disease has been approved by the FDA as of July 2019. The FDA has approved the direct-to-consumer panel test by 23andme that includes a single nucleotide polymorphism in HLA-DQA1 (FDA, 2017).

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
81376	HLA Class II typing, low resolution (eg, antigen equivalents); one locus (eg, HLA-DRB1, -DRB3/4/5, -DQB1, -DQA1, -DPB1, or -DPA1), each
81377	HLA Class II typing, low resolution (eg, antigen equivalents); one antigen equivalent, each
81382	HLA Class II typing, high resolution (ie, alleles or allele groups); one locus (eg, HLA-DRB1, -DRB3/4/5, -DQB1, -DQA1, -DPB1, or -DPA1), each
81383	HLA Class II typing, high resolution (ie, alleles or allele groups); one allele or allele group (eg, HLA-DQB1*06:02P), each
82784	Gammaglobulin (immunoglobulin); IgA, IgD, IgG, IgM, each
83516	Immunoassay for analyte other than infectious agent antibody or infectious agent antigen; qualitative or semiquantitative, multiple step method
86231	Endomysial antibody (EMA), each immunoglobulin (Ig) class
86255	Fluorescent noninfectious agent antibody; screen, each antibody
86256	Fluorescent noninfectious agent antibody; titer, each antibody
86258	Gliadin (deamidated) (DGP) antibody, each immunoglobulin (Ig) class
86364	Tissue transglutaminase, each immunoglobulin (Ig) class
88305	Level IV - Surgical pathology, gross and microscopic examination, colon biopsy

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

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Revision History

Revision Date	Summary of Changes
09/06/2023	Reviewed and Updated: Updated the background, guidelines and recommendations, and evidence-based scientific references. Literature review necessitated the following changes to coverage criteria: All CC except CC9 and CC14 edited for clarity and consistency. Addition of new CC1 and CC2: "1) For individuals who have been diagnosed with celiac disease and who are IgA sufficient, serologic testing with IgA anti-tissue transglutaminase (TTG) MEETS COVERAGE CRITERIA at the following intervals: a) At the first follow-up visit 3-6 months after diagnosis. b) Every 6 months until normalization of anti-TTG levels has occurred. c) Every 12-24 months thereafter. 2) For individuals who have been diagnosed with celiac disease and who are IgA deficient, testing for IgG endomysial antibodies, IgG deamidated gliadin peptide, or IgG TTG MEETS COVERAGE CRITERIA at the following intervals: a) At the first follow-up visit 3-6 months after diagnosis. b) Every 6 months until normalization of IgG levels has occurred. c) Every 12-24 months thereafter." Former CC4, now CC6, broken into subcriteria for clarity. Now reads: "6) Testing for IgA and IgG antibodies to deamidated gliadin peptides MEETS COVERAGE CRITERIA in any of the following situations: a) For individuals under 2 years of age with a clinical suspicion of celiac disease (see Note 1). b) For individuals over 2 years of age as a substitute for anti-TTG testing." New CC11: "11) For asymptomatic individuals not at an increased risk for developing celiac disease (see Note 1), testing for celiac disease DOES NOT MEET COVERAGE CRITERIA."

Cervical Cancer Screening

Policy Number: AHS – G2002 – Cervical Cancer Screening	Prior Policy Name and Number, as applicable:
Initial Presentation Date: 09/18/2015 Effective Date: 02/01/2025	

Policy Description

Cervical cancer screening detects cervical precancerous lesions and cancer through cytology, human papillomavirus (HPV) testing, and if needed, colposcopy (Feldman et al., 2024). The principal screening test to detect cancer in asymptomatic individuals with a cervix is the Papanicolaou (Pap) smear. It involves cells being scraped from the cervix during a pelvic examination and spread onto a slide. The slide is then sent to an accredited laboratory to be stained, observed, and interpreted (Feldman & Crum, 2023).

Human papilloma virus (HPV) has been associated with development of cervical intraepithelial neoplasia, and FDA approved HPV tests detecting the presence of viral DNA from high risk strains have been developed and validated as an adjunct primary cancer screening method (Feldman & Crum, 2023).

For additional information on testing for HPV, please refer to AHS-G2157-Diagnostic Testing of Common Sexually Transmitted Infections.

Terms such as male and female are used when necessary to refer to sex assigned at birth.

Related Policies

Policy Number	Policy Title
AHS-G2157	Diagnostic Testing of Common Sexually Transmitted Infections

AHS-M2057	Diagnosis of Vaginitis

Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

The criteria below are based on recommendations by the U.S. Preventive Services Task Force, The National Cancer Institute, NCCN, The American Society for Colposcopy and Cervical Pathology, The American Cancer Society, The American Society for Clinical Pathology, and the American College of Obstetricians and Gynecologists. Within these coverage criteria, "individual(s)" is specific to individuals with a cervix.

1) For immunocompromised or immunosuppressed individuals, **any one** of the following cervical cancer screening techniques **MEETS COVERAGE CRITERIA**:
   a) Annual cervical cytology testing for individuals of all ages.
   b) Co-testing (cervical cytology and high-risk HPV testing) once every 3 years for individuals 30 years of age or older.

2) For individuals 21 to 29 years of age, cervical cancer screening once every 3 years using conventional or liquid-based Papanicolaou (Pap) smears **MEETS COVERAGE CRITERIA**.

3) For individuals 30 to 65 years of age, **any one** of the following cervical cancer screening techniques **MEETS COVERAGE CRITERIA**:
   a) Conventional or liquid-based Pap smear once every 3 years.

4) For individuals who are over 65 years of age **and** who are considered high-risk (individuals with a high-grade precancerous lesion or cervical cancer, individuals with in utero exposure to diethylstilbestrol (DES)), cervical cancer screening at the frequency described in coverage criterion 3 **MEETS COVERAGE CRITERIA**.

5) For individuals who are HPV positive **and** cytology negative, nucleic acid testing for high-risk strains HPV-16 and HPV-18 **MEETS COVERAGE CRITERIA**.

6) For individuals 65 years of age or younger, annual cervical cancer screening by Pap smear or HPV testing **MEETS COVERAGE CRITERIA** in **any** of the following situations:
   a) For individuals who had a previous cervical cancer screen with an abnormal cytology result and/or who was positive for HPV.
   b) For individuals at high risk for cervical cancer (organ transplant, exposure to the drug DES).

7) For all situations not addressed above, cervical cancer screening (cervical cytology, HPV testing) for individuals less than 21 years of age **DOES NOT MEET COVERAGE CRITERIA**.

8) For individuals over 65 years of age who are not considered high-risk **and** who have an adequate screening history, routine cervical cancer screening **DOES NOT MEET COVERAGE CRITERIA**. Adequate screening history is defined as either:
   a) Having three consecutive negative Pap smears.

b) Having two consecutive negative HPV tests within 10 years before cessation of screening, with the most recent test occurring within 5 years.

9) For individuals who have undergone surgical removal of the uterus and cervix and who have no history of cervical cancer or pre-cancer, cervical cancer screening (at any age) **DOES NOT MEET COVERAGE CRITERIA**.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

10) The following **DO NOT MEET COVERAGE CRITERIA**:

a) Other technologies for cervical cancer screening.

Table of Terminology

Term	Definition
ACOG	American College of Obstetricians and Gynecologists
ACS	American Cancer Society
AIS	Adenocarcinoma in situ
ASCCP	American Society for Colposcopy and Cervical Pathology
ASC-H	Atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion
ASCO	American Society of Clinical Oncology
ASCP	American Society of Clinical pathology
ASCUS	Atypical squamous cells of undetermined significance
CIN (2, 3, 3+)	Cervical intraepithelial neoplasia (Grade 2, 3, 3+)
CLIA '88	Clinical Laboratory Improvement Amendments Of 1988
CMS	Centers For Medicare and Medicaid Services
DES	Diethylstilbestrol
DNA	Deoxyribonucleic acid
EASC	European AIDS Clinical Society
FDA	Food and Drug Administration
GvHD	Graft versus/against the host disease
HGSIL/HSIL	High-grade squamous intraepithelial lesion
HHS	Health and Human Services
HIV	Human immunodeficiency virus
HPV	Human papillomavirus infection
HSCT	Hematopoietic stem cell transplantation
IPD	Individual participant data
JAMA	Journal of the American Medical Association
LDTs	Laboratory developed tests
LEEP	Loop electrosurgical excision procedure
LGSIL/LSIL	Low-grade squamous intraepithelial lesion
mRNA	Messenger ribonucleic acid
NAAT	Nucleic acid amplification test
NCCN	National Comprehensive Cancer Network
NCI	National Cancer Institute

Pap	Papanicolaou
PCR	Polymerase chain reaction
RNA	Ribonucleic acid
SGO	Society of Gynecologic Oncology
STIs	Sexually transmitted infections
USPSTF	United States Preventive Services Task Force
VAT	Visual assessment for treatment
VIA	Visual inspection with acetic acid
VIAC	Visual inspection with acetic acid and digital cervicography

Scientific Background

The American Cancer Society estimates that 13,820 new cases of cervical cancer will be diagnosed in 2024 and approximately 4,360 of these individuals will die from the disease (ACS, 2023). To screen for cervical cancer, a Papanicolaou (Pap) test or human papillomavirus (HPV) test is performed. Co-testing with both is also a common clinical practice. To obtain the cell sample for cytology, cells are scraped from both the ectocervix (external surface) and endocervix (cervical canal) during a speculum exam to evaluate the squamocolumnar junction where most neoplasia occur.

Cytological examination can be performed as either a traditional Pap smear where the swab is rolled directly on the slide for observation or as a liquid-based thin layer cytology examination where the swab is swirled in a liquid solution so that the free cells can be trapped and plated as a monolayer on the glass slide. One advantage of the liquid cytology assay is that the same sample can be used for HPV testing whereas a traditional Pap smear requires a second sample to be taken. HPV testing is typically a nucleic acid-based assay that checks for the presence of high-risk types of HPV, especially types 16 and 18. HPV testing can be performed on samples obtained during a cervical exam; furthermore, testing can be performed on samples obtained from a tampon, Dacron or cotton swab, cytobrush, or cervicovaginal lavage (Feldman & Crum, 2023).

Cervical cancer screening recommendations for average-risk individuals generally fall into categories based on an individual's age (William R Robinson, 2024b):

- Age < 21 – It is suggested to not screen for cervical cancer in asymptomatic and immunocompetent patients (as observational studies show a low incidence and benefits may outweigh the harms of false positives).
- Age 21 to 29 – In average patients that are asymptomatic and immunocompetent, the age at which to initiate screening is contested and the ideal testing method varies by guideline. Opinions for expert groups also vary. A preference for cytology (rather than HPV testing) for this subgroup is based on a meta-analysis of randomized trials that revealed higher false positive rates for HPV testing.
- Age 30 to 65 – It is recommended that cervical cancer screening continues in all immunocompetent and asymptomatic individuals with a cervix. The methods range from primary HPV testing every 5 years to co-testing (Pap and HPV testing) every five years; or a Pap test alone every three years.
- Age >65 years – The decision to halt cervical cancer screening in asymptomatic and immunocompetent patients can depend on factors such as prior screening results, life expectancy, and patient preference, but it is suggested to discontinue screening for this subgroup if there has been adequate prior screening.

The above recommendations do not account for special populations such as patients with HIV, immunosuppression, and in utero exposure to diethylstilbestrol (DES). These populations are at greater risk for developing cervical cancer (William R Robinson, 2024b).

The following are the initial screening recommendations for individuals with HIV (William R Robinson, 2024a):

- Initial screening for HIV should occur when HIV is first diagnosed (but at no earlier than 21 years of age).
- Age 21 to 29 – Cervical cytology is the preferred method for screening.
- Age 30 years or older – Cervical cytology or co-testing are both appropriate. However, the use of HPV testing alone (i.e., without co-testing) is NOT recommended for this subgroup.

For patients with HIV in whom initial screening is normal, subsequent screening is categorized based upon method (i.e., cervical cytology, co-testing, colposcopy)(William R Robinson, 2024a):

- Cervical cytology: Those screened with cervical cytology (patients 21 to 29 years and those 30 and older) should have cervical cytology performed every 12 months for a total of three years. If results of three consecutive cytology tests are normal, a follow-up test can occur every three years.
- Co-testing: Those screened with co-testing (30 years and older) should have this co-testing occur every three years.
- Colposcopy: Should not be performed routinely at follow-up visits.
- Screening in the HIV population should occur throughout a patient's lifetime and should not stop at 65 years old (contrasted against the general average patient recommendations, which suggest discontinuing at 65 years old).

Analytical Validity

A study by Marchand et al. (2005) explored the optimal collection technique for Pap testing. Their study occurred in two different cytology labs and 128 clinicians participated in the study over the course of one year. The authors discovered that in conventional Pap testing the sequence of collection—the cytobrush for the endocervix and the spatula for the ectocervix—had no effect on the quality of the assay. Further, 47% of the clinicians who had high levels of absent endocervical cells on their samples used the cytobrush method alone. The authors concluded, "The combination of the Cytobrush (endocervix) and spatula (ectocervix) is superior for a quality Pap smear. The sequence of collection was not important in conventional Pap smears. The broom alone performs poorly" (Marchand et al., 2005).

Urine-based HPV DNA testing as a screening tool would be a less invasive method than cervical examinations and swabs. A study by Mendez et al. (2014) using both urine samples and cervical swabs from 52 patients, however, showed that there was only 76% agreement between the two methodologies. The urine testing correctly identified 100% of the uninfected individuals but only 65% of the infected as compared to the cervical swab controls (Mendez et al., 2014). An extensive meta-analysis of 14 different studies using urinary testing, on the other hand, reported an 87% sensitivity and 94% specificity of the urine-based methodology for all strains of HPV, but the sensitivity for high-risk strains alone was only 77%. The specificity for the high-risk strains alone was reported to be higher at 98%. "The major limitations of this review are the lack of a strictly uniform method for the detection of HPV in urine and the variation in accuracy between individual studies. Testing urine for HPV seems to have good accuracy for the detection of cervical HPV and testing first void urine samples is more accurate

than random or midstream sampling. When cervical HPV detection is considered difficult in certain subgroups, urine testing should be regarded as an acceptable alternative" (Pathak et al., 2014).

Clinical Utility and Validity

The National Cancer Institute (NCI) reports that "Regular Pap screening decreases cervix cancer incidence and mortality by at least 80%" (NCI, 2024). They also note that Pap testing can result in the possibility of additional diagnostic testing, especially in younger individuals, when unwarranted, especially in cases of possible low-grade squamous intraepithelial lesions (LSILs); however, even though 50% of individuals undergoing Pap testing required additional, follow-up diagnostic procedures, only 5% were treated for LSILs. The NCI also reports that "HPV-based screening provides 60% to 70% greater protection against invasive cervical carcinoma, compared with cytology" (NCI, 2024).

A study by Sabeena et al. (2019) measured the utility of urine-based sampling for cervical cancer screening in low-resource settings. The researchers compared 114 samples to determine the accuracy of HPV detection (by polymerase chain reaction (PCR)) in paired cervical and urine samples. Samples were taken from patients previously diagnosed with cervical cancer through histological methods. Of the 114 samples, "HPV DNA was tested positive in cervical samples of 89 (78.1%) and urine samples of 55 (48.2%) patients. The agreement between the two sampling methods was 66.7%" (Sabeena et al., 2019). HPV detection in urine samples had a sensitivity of 59.6% and a specificity of 92%. The authors concluded, "Even though not acceptable as an HPV DNA screening tool due to low sensitivity, the urine sampling method is inexpensive and more socially acceptable for large epidemiological surveys in developing countries to estimate the burden" (Sabeena et al., 2019).

Cervical cancer guidelines published by the National Comprehensive Cancer Network (NCCN) (NCCN, 2024) state that, although the rates of both incidence and mortality of squamous cell carcinoma of the cervix have been declining over the last thirty years, "adenocarcinoma of the cervix has increased over the past 3 decades, probably because cervical cytologic screening methods are less effective for adenocarcinoma." A study in the United Kingdom supports this increase in adenocarcinoma findings because the risk-reduction associated with three yearly screenings was reduced by 75% for squamous carcinoma and 83% for adenosquamous carcinoma, but adenocarcinoma was reduced only by 43% (Sasieni et al., 2009). Another extensive study of more than 900,000 individuals in Sweden showed that PCR-based HPV testing for the high-risk types 16 and 18 is better at predicting the risk of both in situ and invasive adenocarcinoma. The authors conclude, "infections with HPV 16 and 18 are detectable up to at least 14 years before diagnosis of cervical adenocarcinoma. Our data provide prospective evidence that the association of HPV 16/18 with cervical adenocarcinoma is strong and causal" (Dahlstrom et al., 2010).

A report by Chen et al. (2011) reviewed HPV testing and the risk of the development of cervical cancer. Of the 11,923 individuals participating in the study, 86% of those who tested positive for HPV did not develop cervical cancer within ten years. The authors concluded, "HPV negativity was associated with a very low long-term risk of cervical cancer. Persistent detection of HPV among cytologically normal [individuals] greatly increased risk. Thus, it is useful to perform repeated HPV testing following an initial positive test" (Chen et al., 2011).

In 2018, the results of a multi-year cervical cancer screening trial (FOCAL) were published. This randomized clinical trial tested the use of HPV testing alone for detection of cervical intraepithelial neoplasia (CIN) grade 3 or worse (CIN3+). More than 19,000 individuals participated in the study—split between the intervention group (HPV testing alone) and the control group (liquid-based cytology).

Among individuals who underwent cervical cancer screening, the use of primary HPV testing as compared with cytology testing resulted in a significantly lower likelihood of CIN3+ at 48 months. "Further research is needed to understand long-term clinical outcomes as well as cost-effectiveness" (Ogilvie et al., 2018). In a commentary concerning the findings of this trial, the author noted that "multiple randomized trials have shown that primary HPV screening linked to subsequent identification and treatment of cervical precancer is more effective than Pap testing in reducing the incidence of cervical cancer and precancer, at the cost of lower specificity and more false-negative subsequent colposcopic assessments" (Massad, 2018). The author did not address the limitations of the FOCAL study, including that the study concluded prior to seeing what effects, if any, those vaccinated against HPV 16 and HPV 18 would have since the adolescents vaccinated upon FDA approval of the vaccine would not have necessarily been included within the study. They also state that a limitation of the FOCAL trial is "the use of a pooled HPV test for screening, incorporating all carcinogenic HPV types in a single positive or negative result" (Massad, 2018).

Melnikow et al. (2018) performed a review for the USPSTF regarding cervical cancer screening through high-risk (hr) HPV testing. The authors reviewed the following studies: "8 randomized clinical trials (n = 410556), 5 cohort studies (n = 402615), and 1 individual participant data (IPD) meta-analysis (n = 176464)." Primary hr-HPV testing was found to detect cervical intraepithelial neoplasia (CIN) 3+ at an increased rate (relative risk rate ranging from 1.61 to 7.46) in round 1 screening. False positive rates for primary hr-HPV testing ranged from 6.6% to 7.4%, compared with 2.6% to 6.5% for cytology, whereas in cotesting, false-positives ranged from 5.8% to 19.9% in the first round of screening, compared with 2.6% to 10.9% for cytology. Overall, the authors concluded that "primary hrHPV screening detected higher rates of CIN 3+ at first-round screening compared with cytology. Cotesting trials did not show initial increased CIN 3+ detection" (Melnikow et al., 2018).

Bonde et al. (2020) performed a systematic review on the clinical utility of HPV genotyping as a form of cervical cancer screening. Through 16 studies, the researchers concluded that "HPV genotyping can refine clinical management" for individuals "screened through the primary HPV paradigm and the co-testing paradigm by stratifying genotype-specific results and thereby assign those at highest risk for cervical disease to further testing (i.e., colposcopy) or treatment, while designating those with lowest risk to retesting at a shortened interval." After deeming low risk of bias, the review also stated "the overall quality of evidence for CIN 3 or worse risk with negative for intraepithelial lesions or malignancies or low-grade squamous intraepithelial cytology was assessed as moderate; that with atypical squamous cells-undetermined significance and "all cytology" was assessed as high… Human papillomavirus genotyping discriminated risk of CIN 3 or worse to a clinically significant degree, regardless of cytology result" (Bonde et al., 2020).

Between 2010 and 2019, Pry et al. (2021) reviewed 204,225 results from 183,165 study participants across 11 government health facilities in Lusaka, Zambia, as part of the Cervical Cancer Prevention Program in Zambia (CCPPZ). By examining precancerous lesions via visual inspection with acetic acid and digital cervicography (VIAC), they were able to show that the highest odds for screening positive are among individuals aged 20–29 years and that individuals "in the 30–39 years age group had the highest proportion of positive screening results (11·3%) among those with age recorded"; interestingly, however, those "who were HIV-positive and younger than 20 years had more than three times the predictive probability (18·4, 95% CI 9·56–27·32) for being positive compared with [individuals] who were HIV-negative in the same age group (predictive probability 5·5%, 95% CI 3·2–7·8)" (Pry et al., 2021). But while the high proportion of the screen positivity in individuals younger than 20 years old may suggest that individuals "with HIV have earlier disease progression" and that these individuals "should be engaged in screening at a younger age", these data could be the result of "some misalignment between screening

test positivity and neoplastic lesions, as visually, cervicitis and other benign cervical lesions could be mistaken for pre-cancerous disease" or even simply the inherent weaknesses in the test accuracy of the VIAC method ("sensitivity from 25% (95% CI 7–59) to 82% (66–95) and specificity from 74% (64–82) to 83% (77–87)"), warranting further examination (Pry et al., 2021).

Many guidelines call for the cessation of cervical cancer screening after the age of 65; however, Dilley et al. (2021) argues for a reevaluation of recommendations of this ilk, given that 20% of new cervical cancers occur in this group. Moreover, elderly individuals with a cervix are not only more likely to be diagnosed with late-stage cancer, but also receive commensurately worse outcomes and higher mortality rates. The authors point to the use of theoretical modelling and expert opinion as leading drivers of misconceptions about cervical screening harm in older individuals, specifying that while many of the models seek to minimize the harms and costs associated with increased colposcopies, they are remiss in their consideration of the costs and benefits of "the treatment of advanced cancer, such as cold knife conization, radical hysterectomy, pelvic radiation therapy and chemotherapy" and in their interpretation of exiguous data on the benefits and harms of screening after 65. Furthermore, though the existing guidelines suggest that "the guidelines account for the importance of adequate prior screening before cessation of screening," as the majority of cervical cancer cases are diagnosed in individuals who have not been adequately screened, the authors counter that studies have shown that only 25–50% of individuals diagnosed with cervical cancer had "adequate prior screening" before their cancer diagnosis, which will only be further exacerbated as the population continues to age (Dilley et al., 2021).

Qin et al. (2023) studied annual trends in cervical cancer screening-associated services in average-risk women 65 years or older with adequate prior screening. The US Preventative Services Task Force recommends against cervical cancer screening for women 65 years or older with adequate prior screening. Data was collected between 1999 and 2019 from over 15 million (N=15323635) women between the ages of 65 and 114 with Medicare free-for-service coverage. "From 1999 to 2019, the percentage of women who received at least 1 cytology or HPV test decreased from 18.9% (2.9 million women) in 1999 to 8.5% (1.3 million women) in 2019, a reduction of 55.3%; use rates of colposcopy and cervical procedures decreased 43.2% and 64.4%, respectively." Further, "the total Medicare expenditure for all services rendered in 2019 was about $83.5 million." The authors concluded that "while annual use of cervical cancer screening-associated services in the Medicare fee-for-service population older than 65 years has decreased during the last 2 decades, more than 1.3 million women received these services in 2019 at substantial costs" (Qin et al., 2023).

Winer et al. (2023) studied the effectiveness of direct-mail and opt-in approaches for offering HPV self-sampling kits. The kits were offered, by mail or opt-in, to females between the ages of 30 and 64 who had been previously screened, at least three months prior, and were due for their next screening. A total of 31,355 participants were included. Participants were classified in three groups: those due for screening, those overdue for screening, or individuals with unknown history of screening. Withing each group, individuals were randomly assigned to receive usual care, education (usual care plus educational materials about screening), direct mail (usual care, educational materials, and a mailed self-sampling kit), or opt-in (usual care, education, and the option to request a kit). In individuals due for screening, screening completion was 14.1% higher in the direct-mail group than the education group, and 3.5% higher in the opt-in group than the education group. In individuals overdue for screening, screening completion was 16.9% higher in the direct-mail group than the education group. In individuals with unknown history, screening was 2.2% higher in the opt-in group than the education group. The authors concluded that "within a US health care system, direct-mail self-sampling increased cervical cancer

screening by more than 14% in individuals who were due or overdue for cervical cancer screening" and "the opt-in approach minimally increased screening" (Winer et al., 2023).

Guidelines and Recommendations

U.S. Preventive Services Task Force (USPSTF)

The USPSTF updated their recommendations in 2018. The recommendations are outlined in the table below. The USPSTF changed the recommendation concerning women aged 30-65 to now include the possibility of high-risk HPV testing alone once every five years as a screening. They still allow for the possibility of co-testing every five years or for Pap testing alone every three years.

The USPSTF notes certain risk factors that may increase the risk of cervical cancer, such as "HIV infection, a compromised immune system, in utero exposure to diethylstilbestrol, and previous treatment of a high-grade precancerous lesion or cervical cancer." Cytology, primary testing for high-risk HPV alone, or both methods simultaneously may detect the high-risk lesions that are precursors to cervical cancer (USPSTF, 2018).

The USPSTF Summary of Recommendations and Evidence (USPSTF, 2018):

Population	Recommendation	Grade
Women 21 to 65 years of age	For women 21 to 29 years of age, screen for cervical cancer every 3 years with cytology alone. For women 30 to 65 years of age, screen for cervical cancer every 3 years with cytology alone, every 5 years with high-risk (hr) HPV testing alone, or every 5 years with co-testing.	The USPSTF recommends the service. There is high certainty that the net benefit is substantial. Offer or provide this service. Grade A
Women younger than 21, older than 65, who have had adequate prior screening, or who have had had a hysterectomy	Do not screen for cervical cancer.	The USPSTF recommends against the service. There is moderate or high certainty that the service has no net benefit or that the harms outweigh the benefits. Discourage the use of this service. Grade D

In 2017, "The USPSTF concludes that the current evidence is insufficient to assess the balance of benefits and harms of performing screening pelvic examinations in asymptomatic, nonpregnant adult women. (I statement) This statement does not apply to specific disorders for which the USPSTF already recommends screening (i.e., screening for cervical cancer with a Papanicolaou smear, screening for gonorrhea and chlamydia)."

National Comprehensive Cancer Network (NCCN)

Regarding the diagnosis and workup for cervical cancer, the NCCN states that "The earliest stages of cervical carcinoma may be asymptomatic or associated with a watery vaginal discharge and postcoital bleeding or intermittent spotting. Often these early symptoms are not recognized by the patient. Because of the accessibility of the uterine cervix, cervical cytology or Papanicolaou (Pap) smears and

cervical biopsies can usually result in an accurate diagnosis. Cone biopsy (i.e., conization) is recommended if the cervical biopsy is inadequate to define invasiveness or if accurate assessment of microinvasive disease is required… However, cervical cytologic screening methods are less useful for diagnosing adenocarcinoma, because adenocarcinoma in situ affects areas of the cervix that are harder to sample (i.e., endocervical canal)" and that "Workup for these patients with suspicious symptoms includes history and physical examination, complete blood count (CBC, including platelets), and liver and renal function tests" (NCCN, 2024).

The NCCN also remarked that "Persistent HPV infection is the most important factor in the development of cervical cancer. The incidence of cervical cancer appears to be related to the prevalence of HPV in the population…. Screening methods using HPV testing may increase detection of adenocarcinoma," adducing that "In developed countries, the substantial decline in incidence and mortality of SCC of the cervix is presumed to be the result of effective screening and higher human papillomavirus (HPV)-vaccination coverage, although racial, ethnic, and geographic disparities exist" (NCCN, 2024). As such, the NCCN lists chronic, persistent HPV infection along with persistently abnormal Pap smear tests as criteria to be considered for women contemplating hysterectomy.

National Cancer Institute (NCI)

Concerning the use of Pap testing in screening, the NCI recommends: "Based on solid evidence, regular screening for cervical cancer with the Pap test in an appropriate population of women reduces mortality from cervical cancer. The benefits of screening women younger than 21 years are small because of the low prevalence of lesions that will progress to invasive cancer. Screening is not beneficial in women older than 65 years if they have had a recent history of negative test results… Based on solid evidence, regular screening with the Pap test leads to additional diagnostic procedures (e.g., colposcopy) and possible overtreatment for low-grade squamous intraepithelial lesions (LSILs). These harms are greatest for younger women, who have a higher prevalence of LSILs, lesions that often regress without treatment. Harms are also increased in younger women because they have a higher rate of false-positive results. Excisional procedures to treat preinvasive disease has been associated with increased risk of long-term consequences for fertility and pregnancy" (NCI, 2024).

Concerning the use of HPV DNA testing, the NCI states: "Based on solid evidence, screening with an HPV DNA or HPV RNA test detects high-grade cervical dysplasia, a precursor lesion for cervical cancer. Additional clinical trials show that HPV testing is superior to other cervical cancer screening strategies. In April 2014, the U.S. Food and Drug Administration approved an HPV DNA test that can be used alone for the primary screening of cervical cancer risk in women aged 25 years and older… Based on solid evidence, HPV testing identifies numerous infections that will not lead to cervical dysplasia or cervical cancer. This is especially true in women younger than 30 years, in whom rates of HPV infection may be higher" (NCI, 2024).

Concerning cotesting, they recommend: "Based on solid evidence, screening every 5 years with the Pap test and the HPV DNA test (cotesting) in women aged 30 years and older is more sensitive in detecting cervical abnormalities, compared with the Pap test alone. Screening with the Pap test and HPV DNA test reduces the incidence of cervical cancer… Based on solid evidence, HPV and Pap cotesting is associated with more false-positives than is the Pap test alone. Abnormal test results can lead to more frequent testing and invasive diagnostic procedures" (NCI, 2024).

Regarding screening women without a cervix, they recommend: "Based on solid evidence, screening is not helpful in women who do not have a cervix as a result of a hysterectomy for a benign condition" (NCI, 2024).

American Cancer Society (ACS)

The American Cancer Society updated their guidelines for cervical cancer screening for individuals at average risk in 2020. Their recommendations are summarized below:

(Adapted from Table 2 of (Fontham et al., 2020), *Comparison of Current and Previous American Cancer Society (ACS) Guidelines for Cervical Cancer Screening*)

Population	2020 ACS Recommendation
Age 21-24	No screening
Age 25-29	HPV test every 5 years (preferred) HPV/Pap cotest every 5 years (acceptable) Pap test every 3 years (acceptable)
Age 30-65	HPV test every 5 years (preferred) HPV/Pap cotest every 5 years (acceptable) Pap test every 3 years (acceptable)
Age 65 and older	No screening if a series of prior tests were normal

(Fontham et al., 2020).

The American Society for Colposcopy and Cervical Pathology (ASCCP)

In 2019, the ASCCP published guidelines for cervical cancer screening in immunosuppressed women without an HIV infection. The following table was provided by Moscicki et al. (2019):

Table 3. Summary of Cervical Cancer Screening Recommendations for Non-HIV Immunocompromised Women

Risk group category	Recommendation
Solid organ transplant	• Cytology is recommended if younger than 30 y • Co-testing is preferred, but cytology is acceptable if 30 y or older • If using cytology alone, perform annual cervical cytology. If results of 3 consecutive cytology results are normal, perform cytology every 3 y • If using co-testing, perform baseline co-test with cytology and HPV. If result of cytology is normal and HPV is negative, co-testing can be performed every 3 y • If transplant before the age of 21 y, begin screening within 1 y of sexual debut • Continue screening throughout lifetime (older than 65 y). Discontinue screening based on shared discussion regarding quality and duration of life rather than age

Risk group category	Recommendation
Allogeneic hematopoietic stem cell transplant	• Cytology is recommended if younger than 30 y • Co-testing is preferred, but cytology is acceptable if 30 y or older • If using cytology alone, perform annual cervical cytology. If results of 3 consecutive cytology results are normal, perform cytology every 3 y • If using co-testing, perform baseline co-test with cytology and HPV. If result of cytology is normal and HPV is negative, co-testing can be performed every 3 y • If transplant before the age of 21 y, begin screening within 1 y of sexual debut • Continue screening throughout lifetime (older than 65 y). Discontinue screening based on shared discussion regarding quality and duration of life rather than age • For HSCT patients who develop a new diagnosis of genital GVHD or chronic GVHD, resume annual cervical cytology until 3 consecutive normal results at which time perform cytology every 3 y, or perform an initial baseline co-test and, if cytology is normal and HPV is negative, perform co-testing every 3 y
Inflammatory bowel disease on immunosuppressant treatments	• Cytology is recommended if younger than 30 y • Co-testing is preferred, but cytology is acceptable if 30 y or older • If using cytology alone, perform annual cervical cytology. If results of 3 consecutive cytology results are normal, perform cytology every 3 y • If using co-testing, perform baseline co-test with cytology and HPV. If result of cytology is normal and HPV is negative, co-testing can be performed every 3 y • If on immunosuppressant therapy before the age of 21 y, begin screening within 1 y of sexual debut • Continue screening throughout lifetime (older than 65 y). Discontinue screening based on shared discussion regarding quality and duration of life rather than age
Inflammatory bowel disease not on immunosuppressant treatment	• Follow general population screening guidelines
Systemic lupus erythematosus and rheumatoid arthritis on immunosuppressant treatments	• Cytology is recommended if younger than 30 y • Co-testing is preferred, but cytology is acceptable if 30 y or older • If using cytology alone, perform annual cervical cytology. If results of 3 consecutive cytology results are normal, perform cytology every 3 y • If using co-testing, perform baseline co-test with cytology and HPV. If result of cytology is normal and HPV is negative, co-testing can be performed every 3 y • If on immunosuppressant therapy before the age of 21 y, begin screening within 1 y of sexual debut • Continue screening throughout lifetime (older than 65 y). Discontinue screening based on shared discussion regarding quality and duration of life rather than age

Risk group category	Recommendation
Rheumatoid arthritis not on immunosuppressive treatments	• Follow general population screening guidelines
Type 1 diabetes mellitus	• Follow general population screening guidelines

Society of Gynecologic Oncology, American Society for Colposcopy and Cervical Pathology, American College of Obstetricians and Gynecologists, American Cancer Society, American Society of Cytopathology, College of American Pathologists, and the American Society for Clinical Pathology

Since the 2011 joint guidelines issued by the American Cancer Society, American Society for Colposcopy and Cervical Pathology, and American Society for Clinical Pathology Screening concerning cervical cancer screening, additional reports regarding the use of primary hrHPV testing so that representatives from the Society of Gynecologic Oncology, American Society for Colposcopy and Cervical Pathology, American College of Obstetricians and Gynecologists, American Cancer Society, American Society of Cytopathology, College of American Pathologists, and the American Society for Clinical Pathology convened to issue interim clinical guidance in 2015. In the 2011 statement, primary hrHPV testing was not recommended. The 2015 recommendations include:

- "Because of equivalent or superior effectiveness, primary hrHPV screening can be considered as an alternative to current US cytology-based cervical cancer screening methods. Cytology alone and cotesting remain the screening options specifically recommended in major guidelines."
- "A negative hrHPV test provides greater reassurance of low CIN3+ risk than a negative cytology result."
- "Rescreening after a negative primary hrHPV screen should occur no sooner than every 3 years."
- "Primary hrHPV screening should not be initiated prior to 25 years of age."

Moreover, they give the following screening algorithm (Huh et al., 2015):

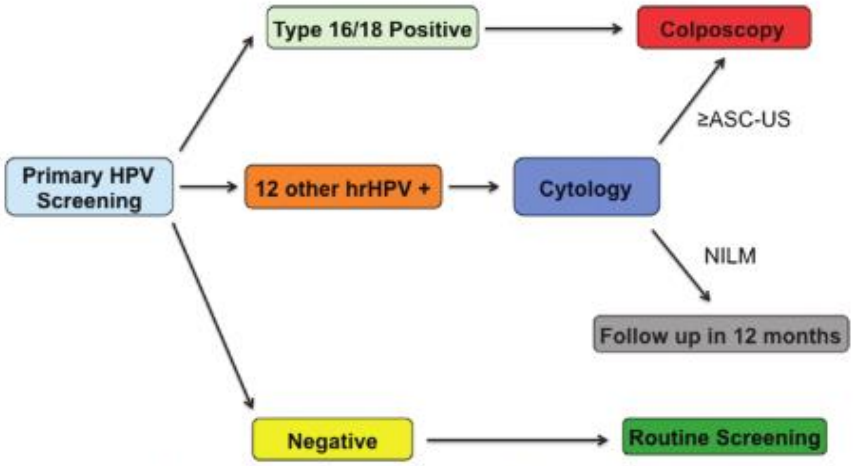

FIGURE 1. Recommended primary HPV screening algorithm. HPV, human papillomavirus; hrHPV, high-risk human papillomavirus; ASC-US, atypical squamous cells of undetermined significance; NILM, negative for intraepithelial lesion or malignancy.

American College of Obstetricians and Gynecologists (ACOG)

In April 2021, the ACOG released a statement withdrawing and replacing the Practice Bulletin No.168 on cervical cancer screening, stating that it will be joining the ASCCP and the SGO "in endorsing the U.S. Preventive Services Task Force (USPSTF) cervical cancer screening recommendations, which replace ACOG Practice Bulletin No.168, *Cervical Cancer Screening and Prevention,* as well as the 2012 ASCCP cervical cancer screening guidelines." This was reaffirmed in 2023 (ACOG, 2021).

In October 2020, the ACOG released "Updated Guidelines for Management of Cervical Cancer Screening Abnormalities." These consensus guidelines are based on risk to determine screening, surveillance, colposcopy, or treatment later in life (ACOG, 2020). In relation to screening, the updated management guidelines state:

1. "Recommendations are based on risk, not results.
    a. Recommendations of colposcopy, treatment, or surveillance will be based on a patient's risk of CIN 3+ determined by a combination of current results and past history (including unknown history). The same current test results may yield different management recommendations depending on the history of recent past test results.
2. Colposcopy can be deferred for certain patients.
    a. Repeat human papillomavirus (HPV) testing or cotesting at 1 year is recommended for patients with minor screening abnormalities indicating HPV infection with low risk of underlying CIN 3+ (e.g., HPV-positive, low-grade cytologic abnormalities after a documented negative screening HPV test or cotest).
3. All positive primary HPV screening tests, regardless of genotype, should have additional reflex triage testing performed from the same laboratory specimen (eg, reflex cytology).
    a. Additional testing from the same laboratory specimen is recommended because the findings may inform colposcopy practice. For example, those HPV-16 positive HSIL cytology qualify for expedited treatment.
    b. HPV 16 or 18 infections have the highest risk for CIN 3 and occult cancer, so additional evaluation (e.g., colposcopy with biopsy) is necessary even when cytology results are negative.

    c. If HPV 16 or 18 testing is positive, and additional laboratory testing of the same sample is not feasible, the patient should proceed directly to colposcopy.
4. Continued surveillance with HPV testing or cotesting at 3-year intervals for at least 25 years is recommended after treatment and initial posttreatment management of histologic HSIL, CIN 2, CIN 3, or AIS. Continued surveillance at 3-year intervals beyond 25 years is acceptable for as long as the patient's life expectancy and ability to be screened are not significantly compromised by serious health issues.
    a. New evidence indicates that risk remains elevated for at least 25 years, with no evidence that treated patients ever return to risk levels compatible with 5-year intervals.
5. Surveillance with cytology alone is acceptable only if testing with HPV or cotesting is not feasible. Cytology is less sensitive than HPV testing for detection of precancer and is therefore recommended more often. Cytology is recommended at 6-month intervals when HPV testing or cotesting is recommended annually. Cytology is recommended annually when 3-year intervals are recommended for HPV or cotesting.
6. Human papilloma virus assays that are Food and Drug Administration (FDA)-approved for screening should be used for management according to their regulatory approval in the United States. (Note: all HPV testing in [the guidelines] refers to testing for high-risk HPV types only).
    a. For all management indications, HPV mRNA and HPV DNA tests without FDA approval for primary screening alone should only be used as a cotest with cytology, unless sufficient, rigorous data are available to support use of these particular tests in management" (ACOG, 2020).

European AIDS Clinical Society (EASC)

The EASC recommends cervical cancer screening (PAP smear or liquid based cervical cytology test) for women over 21 years of age every one to three years. Additionally, the EASC notes "HPV genotype testing may aid PAP/liquid based cervical screening" (EASC, 2023).

United States Department of Health and Human Services (HHS)

The US HHS guidelines for the prevention and treatment of opportunistic infections in adults and adolescents with HIV recommend the following cervical cancer screening:

- "Women with HIV Aged <30 Years:
    - WWH aged 21 to 29 years should have a Pap test following initial diagnosis of HIV.
    - Pap test should be done at baseline and every 12 months (BII).
    - If the results of three consecutive Pap tests are normal, follow-up Pap tests can be performed every 3 years (BII).
    - Co-testing (Pap test and HPV test) is not recommended for women younger than 30 years.
- Women with HIV Aged ≥30 Years:
    - Pap Testing Only
        - Pap test should be done at baseline and every 12 months (BII).
        - If results of 3 consecutive Pap tests are normal, follow-up Pap tests can be performed every 3 years (BII). *Or*
    - Pap Test and HPV Co-Testing
        - Pap test and HPV co-testing should be done at baseline (BII).

        - If result of the Pap test is normal and HPV co-testing is negative, follow up Pap test and HPV co-testing can be performed every 3 years (BII).
        - If the result of the Pap test is normal but HPV co-testing is positive: *Either:*
            - Follow up test with Pap test and HPV co-testing should be performed in 1 year.
            - If the 1-year follow-up Pap test is abnormal, or HPV co-testing is positive, referral to colposcopy is recommended. *Or:*
            - Perform HPV genotyping.
            - If positive for HPV-16 or HPV-18, colposcopy is recommended.
            - If negative for HPV-16 and HPV-18, repeat co-test in 1 year is recommended. If the follow-up HPV test is positive or Pap test is abnormal, colposcopy is recommended: *Or*:
    - Pap Test and HPV16 or HPV16/18 Specified in Co-Testing
        - Pap test and HPV 16 or 16/18 co-testing should be done at baseline (BII).
        - If result of the Pap test is normal, and HPV 16 or 16/18 co-testing is negative, follow-up Pap test and HPV co-testing can be performed every 3 years (BII).
        - If initial test or follow-up test is positive for HPV 16 or 16/18, referral to colposcopy is recommended (BII).
- Primary HPV testing is not recommended (CIII)" (HHS, 2024).

American Society for Clinical Oncology (ASCO)

Resource-stratified recommendations were released in 2022 from the American Society for Clinical Oncology.

For maximal-based resource settings:

- "1.1. In maximal-resource settings, cervical cancer screening with HPV DNA testing should be offered every 5 years from age 25 to 65 years (either self- or clinician-collected). On an individual basis, women may elect to receive screening until age 70 years.
- 1.2. Women who are ≥ 65 years of age who have had consistently negative screening results during past ≥ 15 years may cease screening. Women who are 65 years of age and have a positive result after age 60 should be reinvited to undergo screening 2, 5, and 10 years after the last positive result. If women have received no or irregular screening, they should undergo screening once at 65 years of age, and if the result is negative, exit screening.
- 1.3. If the results of the HPV DNA test are positive, clinicians should then perform triage with reflex genotyping for HPV 16/18 (with or without HPV 45) and/or cytology as soon as HPV test results are known.
- 1.4. If triage results are abnormal (ie, ≥ ASC-US or positive for HPV 16/18 [with or without HPV 45]), women should be referred to colposcopy, during which biopsies of any acetowhite (or suggestive of cancer) areas should be taken, even if the acetowhite lesion might appear insignificant. If triage results are negative (e.g., primary HPV positive and cytology triage negative), then repeat HPV testing at the 12-month follow-up.
- 1.5. If HPV test results are positive at the repeat 12-month follow-up, refer women to colposcopy. If HPV test results are negative at the 12- and 24-month follow-up or negative at any consecutive HPV test 12 months apart, then women should return to routine screening.

- 1.6. Women who have received HPV and cytology co-testing triage and have HPV-positive results and abnormal cytology should be referred for colposcopy and biopsy. If results are HPV positive and cytology normal, repeat co-testing at 12 months. If at repeat testing HPV is still positive, patients should be referred for colposcopy and biopsy, regardless of cytology results.
- 1.7. If the results of the biopsy indicate that women have precursor lesions (CIN2+), then clinicians should offer loop electrosurgical excision procedure (LEEP; if there is a high level of quality assurance [QA]) or, where LEEP is contraindicated, ablative treatments may be offered.
- 1.8. After women receive treatment for precursor lesions, follow-up should consist of HPV DNA testing at 12 months. If 12-month results are positive, continue annual screening; if not, return to routine screening" (ASCO, 2022).

In enhanced-resource settings:

- "2.1. In enhanced-resource settings, cervical cancer screening with HPV DNA testing should be offered to women age 30-65 years, every 5 years (i.e., second screen 5 years from the first) (either self- or clinician-collected).
- 2.2. If there are two consecutive negative screening test results, subsequent screening should be extended to every 10 years.
- 2.3. Women who are ≥ 65 years of age who have had consistently negative screening results during past ≥ 15 years may cease screening. Women who are 65 years of age and have a positive result after age 60 should be reinvited to undergo screening 2, 5, and 10 years after the last positive result. If women have received no or irregular screening, they should undergo screening once at 65 years of age, and if the result is negative, exit screening.
- 2.4. If the results of the HPV DNA test are positive, clinicians should then perform triage with HPV genotyping for HPV 16/18 (with or without HPV 45) and/or reflex cytology.
- 2.5. If triage results are abnormal (ie, ≥ASC-US or positive for HPV 16/18 [with or without HPV 45]), women should be referred to colposcopy, during which biopsies of any acetowhite (or suggestive of cancer) areas should be taken, even if the acetowhite lesion might appear insignificant. If triage results are negative (e.g., primary HPV positive and cytology triage negative), then repeat HPV testing at the 12 month follow-up.
- 2.6. If HPV test results are positive at the repeat 12-month follow-up, refer women to colposcopy. If HPV test results are negative at the 12- and 24-month follow-up or negative at any consecutive HPV test 12 months apart, then women should return to routine screening.
- 2.7. If the results of colposcopy and biopsy indicate that women have precursor lesions (CIN2+), then clinicians should offer LEEP (if there is a high level of QA) or, where LEEP is contradicted, ablative treatments may be offered.
- 2.8. After women receive treatment for precursor lesions, follow-up should consist of HPV DNA testing at 12 months. If 12-month results are positive, continue annual screening; if not, return to routine screening" (ASCO, 2022).

In limited settings:

- "3.1. In limited settings, cervical cancer screening with HPV DNA testing should be offered to women 30 to 49 years of age every 10 years, corresponding to 2 to 3 times per lifetime (either self- or clinician-collected).

- 3.2. If the results of the HPV DNA test are positive, clinicians should then perform triage with reflex cytology (quality assured) and/or HPV genotyping for HPV 16/18 (with or without HPV 45) or with VIA. If institutions are currently using reflex cytology, they should transition from cytology to HPV genotyping.
- 3.3. If cytology triage results are abnormal (i.e. ≥ atypical squamous cells of undetermined significance [ASC-US]), women should be referred to quality assured colposcopy (the first choice, if available and accessible for women who are ineligible for thermal ablation), during which biopsies of any acetowhite (or suggestive of cancer) areas should be taken, even if the acetowhite lesion might appear insignificant. If colposcopy is not available, then perform VAT.
- 3.4. If HPV genotyping or VIA or VAT triage results are positive, then women should be treated. If the results from these forms of triage are negative, then repeat HPV testing at the 12-month follow-up.
- 3.5. If test results are positive at the repeat 12-month follow-up, then women should be treated.
- 3.6. For treatment, clinicians should offer ablation if the criteria are satisfied; if not and resources available, then offer LEEP.
- 3.7. After women receive treatment for precursor lesions, follow-up should consist of the same testing at 12 months" (ASCO, 2022).

Finally, in basic settings:

- "4.1. Health systems in basic settings should move to population-based screening with HPV testing at the earliest opportunity (either self- or clinician-collected). If HPV DNA testing for cervical cancer screening is not available, then VIA should be offered with the goal of developing health systems. Screening should be offered to women 30 to 49 years of age, at least every 10 years (increasing the frequency to every 5 years, resources permitting).
- 4.2. If the results of available HPV testing are positive, clinicians should then perform VAT followed by treatment with thermal ablation and/or LEEP, depending on the size and location of the lesion.
- 4.3. If primary screening is VIA and results are positive, then treatment should be offered with thermal ablation and/or LEEP, depending on the size and location of the lesion.
- 4.4. After women receive treatment for precursor lesions, then follow up with the available test at 12 months. If the result is negative, then women return to routine screening" (ASCO, 2022).

Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

The FDA has approved the APTIMA HPV 16 18/45 Genotype Assay, a nucleic acid amplification test (NAAT), for the qualitative detection of mRNA for HPV 16, 18, and 45 from Gen-Probe Incorporated on October 12, 2012; however, this test cannot distinguish between 18 and 45. Previously, on October 28,

2011, the FDA approved Gen-Probe Incorporated's APTIMA HPV Assay, an NAAT that tests for 14 high-risk types of HPV but is unable to distinguish between the 14 types.

Hologic, Inc. has two FDA-approved HPV NAAT tests—Cervista HPV 16/18 and Cervista HPV HR and GENFIND DNA Extraction Kit. Both were approved on March 12, 2009. The former is a fluorescent, isothermal-based reaction that detects HPV 16 and 18 whereas the latter screens for DNA from the 14 high-risk HPV strains (FDA, 2023a).

The COBAS HPV test by Roche Molecular Systems, Inc. was approved by the FDA on April 19, 2011, as a NAAT for 14 high-risk types of HPV. This test can specifically identify HPV 16 and 18 but cannot distinguish from the other 12 types of HPV. On July 2, 2018, the FDA released an approval order statement (P100020/S025) "for an expansion of the intended use for the FDA-approved cobas HPV Test to include cervical specimens collected in SurePath Preservative Fluid as a specimen type" (FDA, 2023c). This approval allows for the cobas HPV Test to be used as a first-line cervical cancer screening using the SurePath preservative, a medium often used for Pap tests (Rice, 2018). In 2020, the Cobas HPV was FDA approved for use on Cobas 6800/8800 Systems (FDA, 2023b).

On February 12, 2018, the FDA approved the BD Onclarity™ HPV Assay which detects 14 high-risk HPV genotypes including high-risk strains 16 and 18. "The BD Onclarity HPV Assay is a qualitative in vitro test for the detection of Human Papillomavirus in cervical specimens collected by a clinician using an endocervical brush/spatula combination or broom and placed in BD SurePath vial" (FDA, 2018).

For more information regarding HPV, please refer to AHS-G2157 Diagnostic testing of STIs.

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
87623	Infectious agent detection by nucleic acid (DNA or RNA); Human Papillomavirus (HPV), low-risk types (eg, 6, 11, 42, 43, 44)
87624	Infectious agent detection by nucleic acid (DNA or RNA); Human Papillomavirus (HPV), high-risk types (eg, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68)
87625	Infectious agent detection by nucleic acid (DNA or RNA); Human Papillomavirus (HPV), types 16 and 18 only, includes type 45, if performed
88141	Cytopathology, cervical or vaginal (any reporting system), requiring interpretation by physician
88142	Cytopathology, cervical or vaginal (any reporting system), collected in preservative fluid, automated thin layer preparation; manual screening under physician supervision
88143	Cytopathology, cervical or vaginal (any reporting system), collected in preservative fluid, automated thin layer preparation; with manual screening and rescreening under physician supervision
88147	Cytopathology smears, cervical or vaginal; screening by automated system under physician supervision

CPT	Code Description
88148	Cytopathology smears, cervical or vaginal; screening by automated system with manual rescreening under physician supervision
88150	Cytopathology, slides, cervical or vaginal; manual screening under physician supervision
88152	Cytopathology, slides, cervical or vaginal; with manual screening and computer-assisted rescreening under physician supervision
88153	Cytopathology, slides, cervical or vaginal; with manual screening and rescreening under physician supervision
88164	Cytopathology, slides, cervical or vaginal (the bethesda system); manual screening under physician supervision
88165	Cytopathology, slides, cervical or vaginal (the bethesda system); with manual screening and rescreening under physician supervision
88166	Cytopathology, slides, cervical or vaginal (the bethesda system); with manual screening and computer-assisted rescreening under physician supervision
88167	Cytopathology, slides, cervical or vaginal (the bethesda system); with manual screening and computer-assisted rescreening using cell selection and review under physician supervision
88174	Cytopathology, cervical or vaginal (any reporting system), collected in preservative fluid, automated thin layer preparation; screening by automated system, under physician supervision
88175	Cytopathology, cervical or vaginal (any reporting system), collected in preservative fluid, automated thin layer preparation; with screening by automated system and manual rescreening or review, under physician supervision
0500T	Infectious agent detection by nucleic acid (DNA or RNA), Human Papillomavirus (HPV) for five or more separately reported high-risk HPV types (eg, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68) (ie, genotyping)
0502U	Human papillomavirus (HPV), E6/E7 markers for high-risk types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68), cervical cells, branched-chain capture hybridization, reported as negative or positive for high risk for HPV Proprietary test: QuantiVirusTM HPV E6/E7 mRNA Test for Cervical Cancer Lab/Manufacturer: DiaCarta, Inc
G0123	Screening cytopathology, cervical or vaginal (any reporting system), collected in preservative fluid, automated thin layer preparation, screening by cytotechnologist under physician supervision
G0124	Screening cytopathology, cervical or vaginal (any reporting system), collected in preservative fluid, automated thin layer preparation, requiring interpretation by physician
G0141	Screening cytopathology smears, cervical or vaginal, performed by automated system, with manual rescreening, requiring interpretation by physician
G0143	Screening cytopathology, cervical or vaginal (any reporting system), collected in preservative fluid, automated thin layer preparation, with manual screening and rescreening by cytotechnologist under physician supervision
G0144	Screening cytopathology, cervical or vaginal (any reporting system), collected in preservative fluid, automated thin layer preparation, with screening by automated system, under physician supervision
G0145	Screening cytopathology, cervical or vaginal (any reporting system), collected in preservative fluid, automated thin layer preparation, with screening by automated system and manual rescreening under physician supervision

CPT	Code Description
G0147	Screening cytopathology smears, cervical or vaginal, performed by automated system under physician supervision
G0148	Screening cytopathology smears, cervical or vaginal, performed by automated system with manual rescreening
G0476	Infectious agent detection by nucleic acid (DNA or RNA); human papillomavirus (HPV), high-risk types (eg, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68) for cervical cancer screening, must be performed in addition to pap test
P3000	Screening Papanicolaou smear, cervical or vaginal, up to three smears, by technician under physician supervision
P3001	Screening Papanicolaou smear, cervical or vaginal, up to three smears, requiring interpretation by physician
Q0091	Screening Papanicolaou smear; obtaining, preparing and conveyance of cervical or vaginal smear to laboratory

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

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Revision History

Revision Date	Summary of Changes
09/04/2024	Reviewed and Updated: Updated the background, guidelines and recommendations, and evidence-based scientific references. Literature review necessitated the following changes to coverage criteria: CC1 edited to include immunocompromised, now reads: "1) For immunocompromised or immunosuppressed individuals, any one of the following cervical cancer screening techniques MEETS COVERAGE CRITERIA:" Results in removal of "immunocompromised individuals" from CC4 and CC6.b., as it conflicts with the updated societal recommendations outlined in CC1. CC1.a. changed from individuals under 30 to individuals of all ages. Previously read: "a) Annual cervical cytology testing for individuals less than 30 years of age." Now reads: "a) Annual cervical cytology testing for individuals of all ages." Added "high-risk" to CC1.b. for clarity of appropriate testing, now reads: "b) Co-testing (cervical cytology and high-risk HPV) once every 3 years for individuals 30 years of age or older."

	Added "(DES)" to CC4 to define the initialism that is repeated later in CC6b. For clarity on allowed test type for high-risk HPV, "nucleic acid" added to CC5. Now reads: "5) For individuals who are HPV positive and cytology negative, nucleic acid testing for high-risk strains HPV-16 and HPV-18 MEETS COVERAGE CRITERIA." Added "For individuals 65 years of age or younger," to CC6. Now reads: "6) For individuals 65 years of age or younger, annual cervical cancer screening by Pap smear or HPV testing MEETS COVERAGE CRITERIA in any of the following situations:" Added CPT code 0502U (effective date 10/1/2024)

Colorectal Cancer Screening

Policy Number: AHS – G2181 – Colorectal Cancer Screening	Prior Policy Name and Number, as applicable:
Initial Presentation Date: 03/01/2023 Revision Date: 03/06/2024	

Policy Description

Colorectal cancer (CRC) is the term used to describe the development of cancer in the colon or the rectum. Colon cancer and rectal cancer are often grouped together because the two diseases share similar characteristics and features.

Screening is key in detecting colorectal cancer early and has a major impact on colorectal cancer incidence and mortality rates. Screening for colorectal cancer occurs through a preventive visit with a healthcare provider who provides an individual risk assessment.

Related Policies

Policy Number	Policy Title
AHS-M2026	Testing for Colorectal Cancer Management

Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in Section VII of this policy document.

1) For asymptomatic individuals 45 to 75 years of age, annual screening for colorectal cancer

   with a fecal immunochemical test (FIT) (preferred) **or** a guaiac fecal occult blood test (gFOBT) **MEETS COVERAGE CRITERIA**.

2) The use of FIT-DNA (Cologuard) for colorectal cancer screening **MEETS COVERAGE CRITERIA** once every 3 years.

3) The use of methylated Septin 9 (ColoVantage) for colorectal cancer screening **DOES NOT MEET COVERAGE CRITERIA**.

4) For average risk, asymptomatic individuals over 75 years of age, colorectal cancer screening **DOES NOT MEET COVERAGE CRITERIA**.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of a patient's illness.

5) Colorectal cancer screening using **any** of the following techniques **DOES NOT MEET COVERAGE CRITERIA**:

   a) Screening for anal cytological abnormalities (anal pap smear).

   b) Screening for anal HPV infection for individuals under 30 years of age.

Table of Terminology

Term	Definition
AA	Advanced adenoma
ACA	The Patient Protection and Affordable Care Act
ACPM	American College of Preventive Medicine
ACS	American Cancer Society
CDC	Centers for Disease Control and Prevention
CLIA '88	Clinical Laboratory Improvement Amendments of 1988
CMS	Centers for Medicare and Medicaid Services
CRC	Colorectal cancer
CT	Computerized tomography
CUC	Chronic ulcerative colitis
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
FAP	Familial adenomatous polyposis
FDA	Food and Drug Administration
FIT	Fecal immunochemical test
FIT-DNA	Fecal immunochemical test plus DNA test (multi-target)
gFOBT	Guaiac fecal occult blood test
HNPCCC	Hereditary non-polyposis colorectal cancer syndrome
IBD	Inflammatory bowel disease
IOM	Institute of Medicine
KRAS	*KRAS proto-oncogene, GTPase*

MAP	MYH-associated polyposis
MSTF	U.S. Multi-Society Task Force on Colorectal Cancer
NRAS	*NRAS proto-oncogene, GTPase*
PCR	Polymerase chain reaction
USPSTF	U.S. Preventive Services Task Force

Scientific Background

Colorectal cancer (CRC) describes cancer that develops in the colon or rectum. The etiology of colorectal cancer involves a combination of genetic and environmental risk factors. Approximately 75% of patients diagnosed with CRC have a negative family history for colorectal cancer. However, the lifetime risk of developing colorectal cancer increases when an individual has a first-degree relative who was diagnosed under 50 years of age, as well as with other positive family history factors such as two or more affected family members (Kuipers et al., 2015).

Colorectal cancer is a predominant cancer that accounts for 10% of cancer-related mortality in western countries (Kuipers et al., 2015) and is the third leading cause of cancer-related deaths in the United States (Shaukat et al., 2021). For the year 2022, the American Cancer Society (ACS) estimated 106,180 new cases of colon cancer and 44,850 new cases of rectal cancer. Overall, the lifetime risk of developing colorectal cancer is about 1 in 23 (4.3%) for men and 1 in 25 (4.0%) for women (ACS, 2022).

A colorectal cancer screen is typically performed after a risk factor assessment and during an annual wellness visit. Screening efforts focus on finding and removing adenomas and detecting early-stage colorectal cancer. Available screening modalities include CT colonography and stool-based testing (Shaukat et al., 2021). During an annual checkup, providers review an individual's personal history and family history, perform a physical examination, and run a battery of tests.

The types and number of tests performed can vary widely. Several tests for CRC screening are available. These screening tests are designed to detect colorectal cancer and to look for any signs of adenomatous polyps. Stool-based tests detect hemoglobin in blood that comes from a lesion or DNA alterations suggestive of malignancy (Doubeni, 2023).

A fecal immunochemical test (FIT) directly measures hemoglobin in the stool; a patient provides a sample and places it in a specimen collection kit, after which the sample is returned to the lab for processing within 24 hours of collection. FIT tests generate a quantitative result or a qualitative test result and require only one sample, rather than the three days of consecutive sample collection for guaiac-based fecal occult blood tests (gFOBT) (Doubeni, 2023). Quantitative FIT tests–as compared to qualitative FIT tests– are more standardized, produce more consistent results, and have a higher PPV (Doubeni, 2023).

According to the USPSTF, the FIT test has several advantages (one of which is patient convenience) that lead it to be preferred in usage as compared to gFOBT tests. The USPSTF notes that "the fecal immunochemical test (FIT), as a direct measure of human hemoglobin in stool has a number of advantages relative to conventional FOBT and is increasingly used relative to that test" (Robertson et al., 2017). In addition to convenience of use, when compared with gFOBT screening, screening using FIT shows higher detection rates for CRC and advanced adenomas. FIT is also more sensitive than gFOBT for colon lesions (Robertson et al., 2017). Higher sensitivity and higher screening participation rates for FIT contribute to its rate of clinical usage.

A guaiac-based fecal occult blood test is another stool-based test. gFOBT testing detects hemoglobin by turning guaiac reagent-impregnated paper blue through a peroxidase reaction. Hemoglobin identification is necessary to detect any bleeding that may come from a colon lesion. Testing involves a test "card" that is received from a physician's office or through the mail. These test cards are used for three consecutive bowel movements to collect a sample on the card; the cards are mailed into the laboratory for analysis. Several randomized trials have shown that gFOBT screening is effective at reducing CRC mortality. Guidelines recommend providers and laboratories who provide gFOBT screening use only highly sensitive guaiac reagents. One highly sensitive agent is the Hemoccult SENSA, with a reported sensitivity for CRC of 64 to 80 percent, whereas sensitivity for nonrehydrated Hemoccult II tested markedly lower at 25 to 38 percent. Two disadvantages of gFOBT screening should be noted: (1) the sensitivity of gFOBT for advanced adenomas is "substantially less than for CRC" (Doubeni, 2023) and (2) the detection rate for colon lesions on the right side is lower than the detection rate for left-sided lesions.

A multi-target stool DNA test (FIT-DNA) is a composite test made up of a fecal immunochemical test (FIT) and a DNA test that analyzes DNA alterations. Multi-target stool DNA tests are known by a variety of acronyms: sDNA-FIT, MT-sDNA, or FIT-DNA. In the United States, the test is also sometimes listed by its proprietary name: Cologuard. FIT-DNA tests are comprised of molecular assays to test for DNA (*KRAS* mutations); a gene amplification technique to test for methylation markers that arise from colorectal neoplasia; and an immunochemical assay (FIT) to test for hemoglobin, which may be found in blood due to colorectal lesions. The FIT-DNA test procedure involves the patient collecting a stool sample in a specimen collection kit. The collection kit is mailed into the company for testing and should arrive within a 72-hour period after the stool was collected. As of 2022, there are currently no randomized trial results of multi-target sDNA screening for colorectal cancer but there are comparison studies of other screening strategies against multi-target sDNA (Doubeni, 2023).

Proprietary Tests

Cologuard™

Cologuard™ by Exact Sciences Corporation is a test intended to screen adults of either sex, 45 years of age and older, who are at average risk for colorectal cancer (Sciences, 2024). It is intended for the "qualitative detection of colorectal neoplasia associated DNA markers and for the presence of occult hemoglobin in human stool." However, it is not a replacement for diagnostic colonoscopy or surveillance colonoscopy in high-risk individuals, "including patients with a personal history of colorectal cancer and adenomas; have had a positive result from another colorectal cancer screening method within the last 6 months; have been diagnosed with a condition associated with high risk for colorectal cancer such as IBD, chronic ulcerative colitis, Crohn's disease; or have a family history of colorectal cancer, or certain hereditary syndromes" (Sciences, 2024). The proprietors also state that "Positive Cologuard results should be referred to colonoscopy. A negative Cologuard test result does not guarantee absence of cancer or advanced adenoma. Following a negative result, patients should continue participating in a screening program at an interval and with a method appropriate for the individual patient" and that repeat testing has not been evaluated or established (Sciences, 2024).

Imperiale et al. (2014) investigated the screening performance of Cologuard (a noninvasive multi-target DNA test) as compared with a fecal immunochemical test (FIT). Of the 9,989 individuals enrolled in the study, colonoscopy results (as the reference standard) confirmed that 65 individuals (0.7%) had colorectal cancer and 757 (7.6%) had "advanced precancerous lesions." The DNA multi-target stool test used in the study was comprised of a quantitative molecular assay (the assay analyzed *KRAS* mutation,

aberrant *NDRG4* and *BMP3* methylation, and β-actin) and a hemoglobin immunoassay. Multi-target stool DNA testing evidenced specificity of 86.6% for individuals with nonadvanced or negative findings. The sensitivity for detecting advanced precancerous lesions with FIT was 42.4%. Specificity was 94.9% for FIT among participants with nonadvanced or negative findings (P < 100). According to the authors, "The sensitivity of the DNA test for the detection of both colorectal cancer (92.3%) and advanced precancerous lesions (42.4%) exceeded that of FIT by an absolute difference of nearly 20 percentage points. This difference may be attributed to the DNA marker and algorithm components of the test since the test performance of the hemoglobin immunoassay component of the DNA test was nearly identical to that of FIT." In conclusion, the authors noted "the numbers of persons who would need to be screened to detect one cancer were 154 with colonoscopy, 166 with DNA testing, and 208 with FIT" and that "in asymptomatic persons at average risk for colorectal cancer, multi-target stool DNA testing detected significantly more cancers than did FIT but had more false positive results" (Imperiale et al., 2014).

Colovantage®

Colovantage® by Clinical Genomics is a plasma-based test that is used to screen for colorectal cancer and to detect colorectal disease. The test detects circulating methylated DNA from the *SEPT9* gene which is a part of cytokinesis and cell control. The ColoVantage test has yet to be clinically validated as a screening test, but a few small studies are available on this type of test. Grützmann et al. (2008) performed two case-control studies as a part of validation study on *Septin 9* DNA methylation in plasma for screening purposes. The authors used a PCR assay for analysis of *SEPT9*; The samples included 354 samples (252 CRC, 102 controls). A separate study validated the initial one with a blinded, independent study of 309 samples (126 CRC, 183 controls). The use of a *SEPT9* to classify the samples resulted in detection in 120/252 CRCs (48%) and 7/102 (7%) controls; the second case-study resulted in 73/126 CRCs detected (58%) and 18/183 control samples (10%) testing positive for *SEPT9*, validating the initial results. The rate of polyp detection (>1cm) was approximately 20%. According to the authors, "inclusion of an additional measurement replicate increased the sensitivity of the assay in the testing set to 72% while maintaining 90% specificity" (Grützmann et al., 2008).

Shield™

From Guardant Health, Inc., the Shield™ blood-based colorectal cancer screening test "uses a multimodal approach, integrating genomics, epigenomics and proteomics, to detect colorectal cancer signals in the bloodstream, including DNA that is shed by tumors, called circulating tumor DNA (ctDNA)." As an LDT, Shield™ is "intended to be complementary to and not a replacement for current recommended CRC screening methods" (Guardant Health, 2022b).

In 2022, Guardant announced positive results from the ECLIPSE (Evaluation of ctDNA LUNAR Assay In an Average Patient Screening Episode) study in the evaluation of its blood test for the detection of colon cancer in average-risk individuals. This patient registrational study had over 20,000 patients, and Guardant reports that "The test demonstrated 83% sensitivity for the detection of colorectal cancer with specificity of 90%," and may "pave the way for first potential FDA-approved and Medicare-reimbursed blood test for colorectal cancer screening" (Guardant Health, 2022a).

Analytical Validity

Burch et al. (2007) reported on the accuracy of guaiac testing as compared to immunochemical fecal occult blood tests (FOBTs) for the detection of colorectal cancer in an average-risk screening population.

Of the 59 studies evaluated for analytical validity, 33 evaluated guaiac FOBTs and 35 analyzed immunochemical FOBTs. The results showed sensitivities for the detection of all neoplasms ranged from 6.2% to 83.3% for guaiac tests. Specificity ranged from 98.0% to 98.4% for guaiac tests. Sensitivity ranged from 5.4% to 62.6% for immunochemical FOBTs while specificity ranged from 94.3%-98.5% for immunochemical FOBTs (Burch et al., 2007). Sensitivities were also higher for the detection of CRC and lower for adenomas in both the diagnostic cohort and diagnostic case-control studies for both guaiac and immunochemical FOBTs. Of the immunochemical FOBTs, the Immudia HemSp test was the most accurate, but there was "no clear evidence" to prefer either guaiac or immunochemical FOBTs (one over the other) (Burch et al., 2007).

Shapiro et al. (2017) enrolled 1,006 asymptomatic individuals in a study. Participants were 50-75 years of age and had been recommended for a screening colonoscopy (based on colonoscopy screening recommendations). The performance of each test was analyzed, with colonoscopy results used as the reference standard. The InSure FIT test had the highest sensitivity for detecting advanced colorectal neoplasia at 26.3%. The OC FIT-CHEK had a 15.1% sensitivity value. The Hemoccult II SENSA had a test sensitivity value of 7.4%. Statistically, the InSure FIT was more sensitive than the other two tests. Specificity ranged in value from 96.8% to 98.6%. The authors concluded that some FITs were more sensitive than others, but that the results should be confirmed in larger populations (Shapiro et al., 2017).

Kisiel et al. (2022) analyzed the performance of a multi-target stool DNA (mt-sDNA) test that combines the detection of methylation DNA markers (MDMs), *KRAS* mutations and fecal hemoglobin. This verification study included 777 samples – 210 cases and 567 controls. The average age of participants in the study was 65.5 years. The results of the study showed a sensitivity of 95.2% for colorectal cancer (CRC) and a sensitivity of 57.2% for advanced precancerous lesions (APL). Specificity for CRC and advanced precancerous lesions was 89.8% (that is, no CRC or advanced precancerous lesions). A specificity of 92.4% for neoplasia was calculated. Through sub-group analyses, a sensitivity for early-stage CRC of 93.4% at stage I and 94.2% at stage II were determined (Kisiel et al., 2022).

Clinical Utility and Validity

High-sensitivity gFOBTs and FIT tests have been involved in repeated randomized controlled trials for validity and have been shown to reduce colorectal cancer mortality (USPSTF, 2021b).

Faivre et al. (2004) investigated whether a benefit to FOBTs could be ascertained within countries that already had a high performance in the diagnosis and management of colorectal cancer. There were 91,199 individuals ages 45-74 years old who participated in the study. Individuals were allocated to either FOBT screening or no screening. Participants were followed up on for over eleven years. The results of the study showed positivity rates of 2.1% initially and 1.4% on average in subsequent rounds of screening (six screenings were performed over eleven years). Overall CRC mortality was "significantly lower in the screening population compared with the control population (mortality ratio, 0.84; 95% confidence interval)." The authors concluded that "biannual screening by FOBTs could reduce CRC mortality" (Faivre et al., 2004).

Kim et al. (2021) studied the usage of colonoscopy and FIT testing for CRC detection using FIT claims data along with colonoscopy data from the Korean National Health Insurance system over a period of eleven years. Over 61,221 patient records (of individuals newly diagnosed with colorectal cancer) comprised the data used for the study. Another 306,099 individuals who did not have colorectal cancer were used as a control group. Through multivariable logistic regression models, the authors found an

association between colonoscopy and reduced subsequent colorectal cancer risk (adjusted odds ratio of 0.29). Between colonoscopy and distal CRC, there was an even stronger association than with proximal CRC (0.24 vs 0.47). FIT tests were associated with a colorectal cancer risk odds ratio of 0.74. The authors concluded that FIT testing showed less risk reduction than colonoscopy. However, "as the frequency of cumulative FIT assessments increased, the association with CRC prevention became stronger" (Kim et al., 2021).

Guidelines and Recommendations

U.S. Preventive Services Task Force (USPSTF)

The USPSTF provides recommendations regarding clinical preventive services such as screening and counseling. The task force is comprised of an independent panel of experts in primary care and prevention that further specialize in numerous fields. Recommendations are segmented primarily based on factors such as age, gender, and pregnancy status. The USPSTF assigns one of five letter grades to a recommendation (A, B, C, D, or I). The cost of a preventive service is not considered when grading a practice. Furthermore, the recommendations only apply to people who are asymptomatic for a given condition (USPSTF, 2024).

The below chart represents screening recommendations from the USPSTF for adults.

Topic	Date	Grade	Recommendation
Colorectal cancer screening: Adults 45-49 years old (USPSTF, 2021a)	May 2021	B	Recommends screening for colorectal cancer in adults aged 45 to 49 years.
Colorectal cancer screening: Adults 50-75 years old (USPSTF, 2021a)	May 2021	**A**	Recommends screening for colorectal cancer in all adults aged 50 to 75 years.
Colorectal cancer screening: Adults 76-85 years old (USPSTF, 2021a)	May 2021	**C**	Recommends offering screening selectively for colorectal cancer in adults aged 76 to 85 years. Evidence indicates that the benefit of such screening in this age group is small. Clinicians should consider the patient's overall health, prior screening history, and preferences.

The USPSTF provides frequency and efficacy information on available screening methods (USPSTF, 2021b):

Screening method[a]	Frequency[b]	Evidence of efficacy	Other considerations
Stool-based tests			
High-sensitivity gFOBT	Every year	• Evidence from RCTs that gFOBT reduces colorectal cancer mortality • High-sensitivity versions (eg, Hemoccult SENSA) have superior test performance characteristics	• Harms from screening with gFOBT arise from colonoscopy to follow up abnormal gFOBT results

		than older tests (eg, Hemoccult II), although there is still uncertainty about the precision of test sensitivity estimates. Given this uncertainty, it is unclear whether high-sensitivity gFOBT can detect as many cases of advanced adenomas and colorectal cancer as other stool-based tests	• Requires dietary restrictions and three stool samples • Requires good adherence over multiple rounds of testing • Does not require bowel preparation, anesthesia, or transportation to and from the screening examination (test is performed at home)
FIT	Every year	• Evidence from 1 large cohort study that screening with FIT reduces colorectal cancer mortality • Certain types of FIT have improved accuracy compared with gFOBT and HSgFOBT (20 µg hemoglobin per gram of feces threshold was used in the CISNET modeling)	• Harms from screening with FIT arise from colonoscopy to follow up abnormal FIT results • Can be done with a single stool sample • Requires good adherence over multiple rounds of testing • Does not require bowel preparation, anesthesia or sedation, or transportation to and from the screening examination (test is performed at home)
sDNA-FIT	Every 1 to 3[c] y	• Improved sensitivity compared with FIT per 1-time application of screening test • Specificity is lower than that of FIT, resulting in more false-positive results, more follow-up colonoscopies, and more associated adverse events per sDNA-FIT screening test compared with per FIT test • Modeling suggests that screening every 3 y does not provide a favorable (ie, efficient) balance of benefits and harms compared with other stool-based screening options (ie, annual FIT or sDNA-FIT every 1 or 2 y) • Insufficient evidence about appropriate longitudinal follow	• Harms from screening with sDNA-FIT arise from colonoscopy to follow up abnormal sDNA-FIT results • Can be done with a single stool sample but involves collecting an entire bowel movement • Requires good adherence over multiple rounds of testing • Does not require bowel preparation, anesthesia or sedation, or transportation to and from the screening examination (test is performed at home)

		up of abnormal findings after a negative follow-up colonoscopy • No direct evidence evaluating the effect of sDNA-FIT on colorectal cancer mortality	
Direct visualization tests			
Colonoscopy	Every 10 y	• Evidence from cohort studies that colonoscopy reduces colorectal cancer mortality • Harms from colonoscopy include bleeding and perforation, which both increase with age	• Screening and diagnostic follow-up of positive results can be performed during the same examination • Requires less frequent screening • Requires bowel preparation, anesthesia or sedation, and transportation to and from the screening examination
CT colonography	Every 5 y	• Evidence available that CT colonography has reasonable accuracy to detect colorectal cancer and adenomas • No direct evidence evaluating effect of CT colonography on colorectal cancer mortality • Limited evidence about the potential benefits or harms of possible evaluation and treatment of incidental extracolonic findings, which are common. Extracolonic findings detected in 1.3% to 11.4% of exams; <3% required medical or surgical treatment	• Additional harms from screening with CT colonography arise from colonoscopy to follow up abnormal CT colonography results • Requires bowel preparation • Does not require anesthesia or transportation to and from the screening examination
Flexible sigmoidoscopy	Every 5 y	• Evidence from RCTs that flexible sigmoidoscopy reduces colorectal cancer mortality • Risk of bleeding and perforation but less than risk with colonoscopy • Modeling suggests that it provides fewer life-years gained alone than when combined with FIT or in comparison to other strategies	• Additional harms may arise from colonoscopy to follow up abnormal flexible sigmoidoscopy results • Test availability has declined in the US but may be available in some communities where colonoscopy is less available

Flexible sigmoidoscopy with FIT	Flexible sigmoidoscopy every 10 y plus FIT every year	• Evidence from RCTs that flexible sigmoidoscopy + FIT reduces colorectal cancer mortality • Modeling suggests combination testing provides similar benefits to those of colonoscopy, with fewer complications • Risk of bleeding and perforation from flexible sigmoidoscopy but less than risk with colonoscopy	• Additional potential harms from colonoscopy to follow up abnormal flexible sigmoidoscopy or FIT results • Flexible sigmoidoscopy availability has declined in the US but may be available in some communities where colonoscopy is less available • Screening with FIT requires good adherence over multiple rounds of testing

"[a] To achieve the benefits of screening, abnormal results from stool-based tests, CT colonography, and flexible sigmoidoscopy should be followed up with colonoscopy.
[b] Applies to persons with negative findings (including hyperplastic polyps) and is not intended for persons in surveillance programs. Evidence of efficacy is not informative of screening frequency, with the exception of gFOBT and flexible sigmoidoscopy alone.
[c] As stated by the manufacturer"

American Cancer Society (ACS)

For colorectal cancer (CRC), the ACS recommends screening people at average risk starting at age 45. The ACS notes two options for testing: a stool-based test or a visual exam of the colon and rectum. If the patient is in good health, the ACS recommends that regular screening should continue through age 75. From ages 76-85, the ACS writes that the decision to continue screening should be discussed between patient and provider and considers the patient's preferences, overall health, and screening history. From age 85 onward, a patient should no longer receive colorectal cancer screening. If the patient chooses to be screened with a test other than a colonoscopy, any abnormal result must be followed up with a colonoscopy (ACS, 2022).

The ACS notes the following options for CRC screening using stool: "Fecal immunochemical test every y[ear], High-sensitivity, guaiac-based fecal occult blood test every y[ear] or a multitarget stool DNA test every 3 y[ears]" For structural examination, the ACS notes the following options: "colonoscopy every 10 y[ears], CT colonography every 5 y[ears], or flexible sigmoidoscopy every 5 y[ears] (Wolf et al., 2018).

The American College of Gastroenterology (ACG)

The ACG developed both guidance and a modified Grading of Recommendations, Assessment, Development and Evaluation methodology to evaluate the quality of evidence and strength of recommendations. They used "we recommend" for strong recommendations and "we suggest" for conditional recommendations. The following are CRC screening recommendations:

1. "We recommend CRC screening in average-risk individuals between ages 50 and 75 years to reduce incidence of advanced adenoma, CRC, and mortality from CRC. Strong recommendation; moderate-quality evidence

2. We suggest CRC screening in average-risk individuals between ages 45 and 49 years to reduce incidence of advanced adenoma, CRC, and mortality from CRC. Conditional recommendation; very low-quality evidence
3. We suggest that a decision to continue screening beyond age 75 years be individualized. Conditional recommendation; very low-quality evidence
4. We recommend colonoscopy and FIT as the primary screening modalities for CRC screening. Strong recommendation; low-quality evidence
5. We suggest consideration of the following screening tests for individuals unable or unwilling to undergo colonoscopy or FIT: flexible sigmoidoscopy, multitarget stool DNA test, CT colonography or colon capsule. Conditional recommendation; very low-quality evidence
6. We suggest against Septin 9 for CRC screening. Conditional recommendation, very low-quality of evidence
7. We recommend that the following intervals should be followed for screening modalities: FIT every 1 year, Colonoscopy every 10 years. Strong recommendation; low-quality evidence
8. We suggest that the following intervals should be followed for screening modalities: Multitarget stool DNA test every 3 years, Flexible sigmoidoscopy every 5–10 years, CTC every 5 years, CC every 5 years. Conditional recommendation; very low-quality evidence
9. We suggest initiating CRC screening with a colonoscopy at age 40 or 10 years before the youngest affected relative, whichever is earlier, for individuals with CRC or advanced polyp in 1 first degree relative (FDR) at age <60 years or CRC or advanced polyp in ≥2 FDR at any age. We suggest interval colonoscopy every 5 years. Conditional recommendation; very low-quality evidence
10. We suggest consideration of genetic evaluation with higher familial CRC burden (higher number and/or younger age of affected relatives). Conditional recommendation; very low-quality evidence
11. We suggest initiating CRC screening at age 40 or 10 years before the youngest affected relative and then resuming average-risk screening recommendations for individuals with CRC or advanced polyp in 1 FDR at age ≥60 years. Conditional recommendation; very low-quality evidence
12. In individuals with 1 second-degree relative (SDR) with CRC or advanced polyp, we suggest following average-risk CRC screening recommendations. Conditional recommendation; low-quality evidence" (Shaukat et al., 2021).

U.S. Multi-Society Task Force on Colorectal Cancer – American College of Gastroenterology, American Gastroenterological Association, and the American Society for Gastrointestinal Endoscopy

In 2022, Task Force on Colorectal Cancer published an update to their recommendations. The update focused on addressing the age of beginning CRC screening in average-risk individuals as well as the age of stopping CRC screening. The guideline recommends that screening begin at age 45 because there is "increasing disease burden among individuals under age 50, emerging data that the prevalence of advanced colorectal neoplasia in individuals ages 45 to 49 approaches rates in individuals 50 to 59, and modeling studies demonstrate the benefits of screening outweigh the potential harms and costs. For individuals ages 76 to 85, the decision to start or continue screening should be individualized and based on prior screening history, life expectancy, CRC risk, and personal preference. Screening is not recommended after age 85" (Patel et al., 2022).

Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations

(NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

The FDA approved the Epi proColon by Epigenomics AG on April 12, 2016.

"The Epi proColon test is a qualitative in vitro diagnostic test for the detection of methylated Septin 9 DNA in EDTA plasma derived from patient whole blood specimens. Methylation of the target DNA sequence in the promoter region of the SEPT9_v2 transcript has been associated with the occurrence of colorectal cancer (CRC). The test uses a real-time polymerase chain reaction (PCR) with a fluorescent hydrolysis probe for the methylation specific detection of the Septin 9 DNA target. The Epi proColon test is indicated to screen adults of either sex, 50 years or older, defined as average risk for CRC, who have been offered and have a history of not completing CRC screening. Tests that are available and recommended in the USPSTF 2008 CRC screening guidelines should be offered and declined prior to offering the Epi proColon test. Patients with a positive Epi proColon test result should be referred for diagnostic colonoscopy. The Epi proColon test results should be used in combination with physician's assessment and individual risk factors in guiding patient management" (FDA, 2016).

The FDA approved Cologuard™ by Exact Sciences Corporation on August 11, 2014.

"Cologuard is intended for the qualitative detection of colorectal neoplasia associated DNA markers and for the presence of occult hemoglobin in human stool. A positive result may indicate the presence of colorectal cancer (CRC) or advanced adenoma (AA) and should be followed by diagnostic colonoscopy. Cologuard is indicated to screen adults of either sex, 50 years or older, who are at typical average-risk for CRC. Cologuard is not a replacement for diagnostic colonoscopy or surveillance colonoscopy in high risk individuals" (FDA, 2014).

The FDA also lists contraindications for Cologuard, noting that certain populations were not clinically evaluated for Cologuard use. These populations include:

- "Patients with a history of colorectal cancer, adenomas, or other related cancers.
- Patients who have had a positive result from another colorectal cancer screening method within the last 6 months.
- Patients who have been diagnosed with a condition that is associated with high risk for colorectal cancer. These include but are not limited to:
  - Inflammatory Bowel Disease (IBD)
  - Chronic ulcerative colitis (CUC)
  - Crohn's disease
  - Familial adenomatous polyposis (FAP)
  - Family history of colorectal cancer
- Patients who have been diagnosed with a relevant familial (hereditary) cancer syndrome, such as Hereditary non-polyposis colorectal cancer syndrome (HNPCCC or Lynch Syndrome), Peutz-Jeghers Syndrome, MYH-Associated Polyposis (MAP), Gardner's syndrome, Turcot's (or Crail's) syndrome, Cowden's syndrome, Juvenile Polyposis, Cronkhite-Canada syndrome, Neurofibromatosis, or Familial Hyperplastic Polyposis." (FDA, 2014)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
81327	SEPT9 (Septin9) (eg, colorectal cancer) promoter methylation analysis
81528	Oncology (colorectal) screening, quantitative real-time target and signal amplification of 10 DNA markers (KRAS mutations, promoter methylation of NDRG4 and BMP3) and fecal hemoglobin, utilizing stool, algorithm reported as a positive or negative result
82270	Blood, occult, by peroxidase activity (eg, guaiac), qualitative; feces, consecutive collected specimens with single determination, for colorectal neoplasm screening (ie, patient was provided 3 cards or single triple card for consecutive collection)
82274	Blood, occult, by fecal hemoglobin determination by immunoassay, qualitative, feces, 1-3 simultaneous determinations
87624	Infectious agent detection by nucleic acid (DNA or RNA); Human Papillomavirus (HPV), high-risk types (eg, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68)
87625	Infectious agent detection by nucleic acid (DNA or RNA); Human Papillomavirus (HPV), types 16 and 18 only, includes type 45, if performed
88112	Cytopathology, selective cellular enhancement technique with interpretation (eg, liquid based slide preparation method), except cervical or vaginal
0500T	Infectious agent detection by nucleic acid (DNA or RNA), Human Papillomavirus (HPV) for five or more separately reported high-risk HPV types (eg, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68) (ie, genotyping)

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

Evidence-based Scientific References

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Revision History

Revision Date	Summary of Changes
03/06/2024	Reviewed and Updated: Updated the background, guidelines and recommendations, and evidence-based scientific references. Literature review did not necessitate any modifications to coverage criteria. Removed CC1.b., as visualization tests are outside Avalon's scope of management. "1.b) Direct visualization tests: i) Colonoscopy every 10 years. ii) Computerized tomography (CT) every 5 years. iii) Flexible sigmoidoscopy every 5 years. iv) Flexible sigmoidoscopy every 10 years with FIT every year." CC1 edited for clarity and consistency following the removal of CC1.b., now reads "1) For asymptomatic individuals 45 to 75 years of age, annual screening for colorectal cancer with a fecal immunochemical test (FIT) (preferred) or a guaiac fecal occult blood test (gFOBT) MEETS COVERAGE CRITERIA." Removed CC4.c., as endoscopy is outside Avalon's scope of management. "4.c) Colon capsule endoscopy."
03/01/2023	Initial presentation

Coronavirus Testing in the Outpatient Setting

Policy Number: AHS – G2174 – Coronavirus Testing in the Outpatient Setting	Initial Presentation Date: 05/26/2020 Effective Date: 4/1/2025

Policy Description

Human coronaviruses, first characterized in the 1960s, are named based on the spiked proteins located on their surface. As of 2020, seven coronaviruses are known to infect humans. Four, of which—229E, NL63, OC43, and HKU1—are associated with the common cold. MERS-CoV is the coronavirus that causes Middle East Respiratory Syndrome, or MERS. SARS-CoV is the causative agent of Severe Acute Respiratory Syndrome (SARS), and SARS-CoV-2 is the virus that causes coronavirus disease 2019, or COVID-19 (CDC, 2020, 2024a). As of June 1, 2024, the United States had reported that nearly 1.2 million people have died of COVID-19 (CDC, 2024a). Testing for a possible coronavirus infection can include molecular tests, such as nucleic acid-based testing like reverse transcription polymerase chain reaction (RT-PCR); host antibody testing; and antigen testing.

Related Policies

Policy Number	Policy Title
AHS-G2060	Fecal Analysis in the Diagnosis of Intestinal Dysbiosis and Fecal Microbiota Transplant Testing
AHS-G2149	Pathogen Panel Testing
AHS-M2097	Identification of Microorganisms Using Nucleic Acid Probes

Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

This policy only addresses testing for the purpose of medical decision making in the outpatient setting. This policy does not address work, school, state, or federally mandated SARS-CoV-2 testing.

1) Targeted nucleic acid testing (e.g., RT-PCR, rapid molecular tests) for COVID-19 (SARS-CoV-2**) MEETS COVERAGE CRITERIA** in any of the following situations:
   a) For individuals displaying signs and symptoms of possible COVID-19 infection (See Note 1).
   b) For asymptomatic individuals with known exposure to COVID-19, **EXCEPT** when the individual has had a previous COVID-19 infection within the last 90 days.

2) For individuals with signs or symptoms of SARS and who have traveled to endemic areas or who have been exposed to persons with SARS, targeted nucleic acid testing (e.g., RT-PCR) for the detection of severe acute respiratory syndrome (SARS) coronavirus RNA **MEETS COVERAGE CRITERIA**.

3) For individuals with signs or symptoms of Middle East respiratory syndrome (MERS) and who have traveled to endemic areas or who have been exposed to persons with MERS, targeted nucleic acid testing (e.g, RT-PCR) for the detection of MERS coronavirus RNA **MEETS COVERAGE CRITERIA**.

4) To support a diagnosis of multisystem inflammatory syndrome in children (MIS-C) (see Note 2), multisystem inflammatory syndrome in adults (MIS-A) (see Note 3), or post-acute sequelae of SARS-CoV-2 infection (PASC), nucleic acid amplification testing and host antibody serology testing **MEET COVERAGE CRITERIA**.

5) For symptomatic individuals, antigen-detecting diagnostic tests for SARS-CoV-2 (e.g., antigen rapid tests) once every 48 hours **MEET COVERAGE CRITERIA**.

6) For individuals with signs and symptoms of a respiratory tract infection (see Note 4), antigen panel testing of up to 5 antigens **MEETS COVERAGE CRITERIA**.

7) For the diagnosis of SARS-CoV-2 reinfection, whole genome sequencing of paired specimens from distinct lineages (as defined in Nextstrain or GISAID) **DOES NOT MEET COVERAGE CRITERIA**.

8) Antigen panel testing of 6 or more antigens **DOES NOT MEET COVERAGE CRITERIA**.

9) For all other situations not described above, host antibody serology testing **DOES NOT MEET COVERAGE CRITERIA**.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

10) In the outpatient setting, SARS-CoV-2 genotyping **DOES NOT MEET COVERAGE CRITERIA**.

11) For all situations, neutralization antibody testing for SARS-CoV-**2 DOES NOT MEET COVERAGE CRITERIA**.

12) Testing for other endemic coronaviruses, such as 229E, NL63, OC43, and HKU1, **DOES NOT MEET COVERAGE CRITERIA**.

NOTES:

Note 1: Signs and symptoms associated with a possible COVID-19 infection can include fever, cough, fatigue, shortness of breath or difficulty breathing, congestion or runny nose, chills, muscle or body aches, headache, sore throat, new loss of taste or smell, nausea, vomiting, and diarrhea (CDC, 2024g).

Note 2: According the CDC (CDC, 2024e), MIS-C is defined as an illness that is found in a person less than 21 years of age when **all** of the following conditions are met:

- Subjective or documented fever of at least 38°C;
- Clinical severity requiring hospitalization;
- Evidence of systemic inflammation indicated by elevated C-reactive protein (CRP);
- New onset of manifestations in at least **two** of the following categories:
  - Cardiac involvement indicated by **one** of the following:
    - Left ventricular ejection fraction <55%.
    - Coronary artery dilatation, aneurysm, or ectasia.
    - Elevated troponin.
  - Mucocutaneous involvement indicated by **one** of the following:
    - Rash.
    - Inflammation of the oral mucosa.
    - Conjunctivitis or conjunctival injection.
    - Extremity findings (e.g., erythema or edema of the hands or feet).
  - Shock.
  - Gastrointestinal involvement indicated by **one** of the following:
    - Abdominal pain.
    - Vomiting.
    - Diarrhea.
  - Hematologic involvement indicated by **one** of the following:
    - Platelet count <150,000 cells/µL.
    - Absolute lymphocyte count.

Note 3: According to the CDC (CDC, 2024e), MIS-A is defined as an illness that is found in a person 21 years of age or older when all of the following conditions are met:

- Hospitalization for 24 hours or more;
- Subjective or documented fever of at least 38°C for one of the following:
  - 24 or more hours prior to hospitalization.
  - Within the first 3 days of hospitalization.
- No alternative diagnosis (e.g., bacterial sepsis).
- At least **three** of the following (occurring prior to hospitalization or within the first three days of hospitalization), with at least one being a primary clinical criterion:
  - Primary clinical criteria:
    - Severe cardiac illness (e.g., myocarditis, pericarditis, coronary artery dilation/aneurysm, new-onset right or left ventricular dysfunction, 2nd/3rd degree A-V block, ventricular tachycardia).
    - Rash **and** non-purulent conjunctivitis.
  - Secondary clinical criteria:

    - New-onset neurologic signs and symptoms (e.g., encephalopathy in an individuals without prior cognitive impairment, seizures, meningeal signs, peripheral neuropathy including Guillain-Barré syndrome).
    - Shock or hypotension not attributable to medical therapy.
    - Abdominal pain, vomiting, or diarrhea.
    - Thrombocytopenia.
- Evidence of SARS-CoV-2 infection;
- Evidence of systemic inflammation (elevated CRP, ferritin, interleukin-6, erythrocyte sedimentation rate, or procalcitonin).

Note 4: Signs and symptoms of a respiratory tract infection:

- A temperature greater than 102°F
- Pronounced dyspnea
- Tachypnea
- Tachycardia

Table of Terminology

Term	Definition
2019-nCoV	2019 novel coronavirus
AACC	American Association for Clinical Chemistry
AAP	American Academy of Pediatrics
ACE-2	Angiotensin converting enzyme-2
ACR	American College of Rheumatology
ACS	American Chemical Society
Ag-RDTs	Antigen-detecting rapid diagnostic tests
AMA	American Medical Association
APSF	Anesthesia Patient Safety Foundation
ARDS	Acute respiratory distress syndrome
ASA	American Society of Anesthesiologists
ASM	American Society for Microbiology
BAL	Bronchoalveolar lavage
BNP	B-type natriuretic peptide
CARES Act	Coronavirus Aid, Relief, And Economic Security Act
Cas12a	CRISPR associated protein 12a
CBC	Complete blood cell count
CDC	Centers For Disease Control and Prevention
cDNA	Complementary deoxyribonucleic acid
CFR	Code of Federal Regulations
CI	Confidence interval
CLIA	Chemiluminescence enzyme immunoassay
CLIA ‘88	Clinical Laboratory Improvement Amendments Of 1988
CMS	Centers For Medicare & Medicaid Services
COVID-19	Coronavirus disease 2019
CPK	Creatine phosphokinase
CRP	C-reactive protein
CSSE	Center for Systems Science and Engineering

CT	Cycle threshold
cVNT	Competitive neutralization test
DNA	Deoxyribonucleic acid
DPP7	*Dipeptidyl peptidase 7*
ECDC	European Centre for Disease Prevention and Control
ESR	Erythrocyte sedimentation rate
ETS	Emergency temporary standard
EU/EEA	European Union / European Economic Area
EUA	Emergency use authorization
FAQ	Frequently asked questions
FDA	Food and Drug Administration
FET	Field-effect transistor
FIA	Fluorescence immunoassays
Flu SC2	Influenza SARS-CoV-2 (multiplex assay)
FN	False negative
FP	False positive
GISAID	Global initiative on sharing all influenza data
GOLGA3	*Golgi autoantigen, golgin subfamily a, 3*
GRADE	Grading Of Recommendations Assessment, Development, and Evaluation
HCoV	Human coronavirus
HCP	Health care personnel
HCW	Healthcare worker
HHS	Health And Human Services
HKU1	Human coronavirus
HLA	Human leukocyte antigen
HSCT	Hematopoietic stem cell transplant
ICMA	Immunochemiluminometric assay
ICR	Investigative criteria for suspected cases of SARS-CoV-2 reinfection
IDSA	Infectious Diseases Society of America
IFU	Instructions for use
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IL-1	Interleukin 1
IL-6	Interleukin 6
INR	International normalized ratio
IQR	Interquartile range
IVIG	Intravenous immunoglobulin
JAMA	Journal of the American Medical Association
LDH	Lactic acid dehydrogenase
LDTs	Laboratory-developed tests
LFIAs	Lateral flow immunoassays
LoD	Limit of detection
MERS	Middle east respiratory syndrome
MERS-CoV	Middle east respiratory syndrome–related coronavirus
MHRA	Medicines & Healthcare Products Regulatory Agency

MIS-A	Multisystem inflammatory syndrome in adults
MIS-C	Multisystem inflammatory syndrome in children
MMWR	Morbidity And Mortality Weekly Report
MT	Mid-turbinate
N	Nucleocapsid
NAAT	Nucleic acid amplification test
NAb	Neutralizing antibody
NGS	Next-generation sequencing
NIH	National Institutes of Health
NP	Nasopharyngeal
NPA	Negative percent agreement
NT-proBNP	N-terminal pro hormone BNP
NW	Nasopharyngeal wash/aspirate or nasal wash/aspirate
OD	Optical density
OP	Oropharyngeal
opvCRISPR	One-pot visual SARS-CoV-2 detection system
OSHA	Occupational Safety and Health Administration
PASC	Post-Acute Sequelae Of SARS-CoV-2 Infection
PCR	Polymerase chain reaction
PEM	Post-exertional malaise
PHE	Public Health England
PHS Act	Public Health Service Act
POC	Point-of-care
POC/NP	Point of care/near person
PPA	Positive percent agreement
PPE	Personal protective equipment
pro-BNP	Pro hormone B-type natriuretic peptide
PSO	Past symptom onset
PT	Prothrombin time
PTT	Partial thromboplastin time
ptxP	Single-copy promoter target
RADT	Rapid antigen detection test
RBD	Receptor binding domain
RdRp	*Ribonucleic acid-dependent ribonucleic acid polymerase*
RNA	Ribonucleic acid
RP	*Ribonuclease P gene*
RP	Respiratory pathogen
RP2	Respiratory panel 2
RP2.1	Respiratory panel 2.1
RT	Reverse transcriptase
RT-LAMP	Reverse transcription loop-mediated isothermal amplification
RT-PCR	Reverse transcription polymerase chain reaction
SARC	Severe acute respiratory syndrome
SARS-CoV	Severe acute respiratory syndrome- coronavirus
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SF-12	Short form twelve health survey

SHEA	Society for Healthcare Epidemiology of America
SNP	Single nucleotide polymorphism
SOT	Solid organ transplant
ssDNA	Single-stranded deoxyribonucleic acid
sVNT	Surrogate viral neutralization test
$TCID_{50}$	Median tissue culture infective dose
TMA	Transcription-mediated amplification
TMEM189–UBE2V1	PEDS1-UBE2V1 readthrough
TN	True negative
TP	True positive
UCSD	University of California San Diego
VOC	Variant of concern
VUI	Variant under investigation
WGS	Whole genome sequencing
WHO	World Health Organization

Reimbursement

1) AMA standard practice for COVID-19 testing states not to include both the HCPCS and AMA code for the same procedure on the same DOS and that only one code should be used, therefore only one code per date of service will be reimbursed.

2) Specimen collection codes for coronavirus testing are considered incidental and will not be reimbursed.

Scientific Background

On March 11, 2020, the World Health Organization (WHO) declared the novel coronavirus SARS-CoV-2, or COVID-19, a global pandemic (Cucinotta & Vanelli, 2020). COVID-19 is the third recent human coronavirus to be declared an emergency. SARS (Severe Acute Respiratory Syndrome) was recognized as an emergency by the WHO in February 2003 (WHO, 2024b). This outbreak in 2003 resulted in over 8000 cases in 26 different countries. Since 2003, only four limited reoccurrences have been reported according to the WHO—three incidences are due to laboratory accidents (in Taipei and Singapore) and one incident of undetermined source in China (WHO, 2024b). As early as September 2012, another human coronavirus, MERS-CoV, began to spread in the Middle East, causing Middle East Respiratory Syndrome (MERS). Although the WHO did not initially declare MERS an emergency, they have since added MERS to their list of pandemic/epidemic diseases. Since September 2012 and as of the end of October 2021, the WHO reports 2574 laboratory-confirmed cases of MERS with 858 MERS-associated deaths (34.4% fatality rate) in 27 countries (WHO, 2024a).

Unlike the initial SARS and MERS outbreaks that were predominantly regionally contained, COVID-19 became a global pandemic. According to the WHO, as of September 27, 2023, there were more than 770 million confirmed cases of COVID-19 with over 6,959,316 confirmed deaths worldwide (WHO, 2023). Infection from the novel human coronavirus SARS-CoV-2 can result in coronavirus disease 2019 (COVID-19). The WHO reports approximately 15% of individuals with COVID-19 develop severe disease requiring oxygen support while 5% develop "critical disease" with complications such as respiratory failure or multiorgan failure (WHO, 2021b). Older individuals and patients with comorbidities—such as cardiovascular disease, diabetes mellitus, hypertension, chronic lung disease, cancer, chronic kidney

disease, obesity, and smoking—have an increased likelihood of poor outcomes (Gandhi, 2024). Sepsis, multiorgan failure (including the kidney, liver, and heart), pneumonia, and acute respiratory distress syndrome (ARDS) can also occur (WHO, 2021b; Yang et al., 2020). Severe outcomes have been associated with the following laboratory features: lymphopenia, elevated liver enzymes, elevated lactate dehydrogenase (LDH), elevated inflammatory markers (such as CRP and ferritin), elevated D-dimer, elevated prothrombin time (PT), elevated troponin, elevated creatine phosphokinase (CPK), and acute kidney injury (Gandhi, 2024).

Much of what has generated this global pandemic is attributed to the different levels of transmissibility of the SARS-CoV-2 virus compared to SARS-CoV-1 and MERS, which can arise from the viral load. Simply put, viral load is the number of viral particles/virions in a milliliter of blood (Ryding, 2020). The viral load of SARS-CoV-2 "peaks around the time of symptom onset, followed by a gradual decrease to a low level after about 10 days. Regarding the period of high infectiousness, a recent study reported that exposure to an index case within five days of symptom onset confers a high risk of secondary transmission" (Kawasuji et al., 2020). This finding was corroborated by other studies, which found that "SARS-CoV-2 viral load in the upper respiratory tract appeared to peak in the first week of illness, whereas that of SARS-CoV peaked at days 10–14 and that of MERS-CoV peaked at days 7–10;" because SARS-CoV-2 viral load peaks faster, it can be more transmissible earlier in the disease course (Cevik et al., 2021). However, after reaching its peak during symptom onset, the viral load decreases "monotonically" (Kawasuji et al., 2020). If viral loads do not decrease, patients will be more likely to suffer worse outcomes and require hospitalization (Griffin, 2020). Viral load has been found to be either similar among symptomatic and asymptomatic COVID-19 positive individuals, or higher among symptomatic individuals (Kawasuji et al., 2020). Infectiousness of COVID-19 also correlates with shedding, meaning that the viral particles can replicate in an individual and spread in the environment to others. The mean duration of SARS-CoV-2 RNA shedding "was 17.0 days (95% CI 15·5–18·6; 43 studies, 3229 individuals) in upper respiratory tract, 14.6 days (9·3–20·0; seven studies, 260 individuals) in lower respiratory tract, 17.2 days (14·4–20·1; 13 studies, 586 individuals) in stool, and 16.6 days (3·6–29·7; two studies, 108 individuals) in serum samples," with maximum shedding duration reaching "83 days in the upper respiratory tract, 59 days in the lower respiratory tract, 126 days in stools, and 60 days in serum"(Cevik et al., 2021).

In children and adolescents, reports of a multisystem inflammatory syndrome (MIS-C) with similarities to Kawasaki disease and toxic shock syndrome have been linked to COVID-19 (DeBiasi et al., 2020; Jones et al., 2020; Verdoni et al., 2020; WHO, 2020c). Multisystem inflammatory syndrome has also been reported in adults (MIS-A). From June to October 2020, researchers reported 27 cases of MIS-A in the US and UK (Baum, 2020). The case definition of MIS-A includes "(1) hospitalization without evidence of severe respiratory illness (to exclude hypoxia as the cause of the signs and symptoms), (2) extrapulmonary organ system involvement (including hypotension or shock, cardiac dysfunction, arterial or venous thromboembolism, acute liver injury, or dermatologic abnormalities), and (3) laboratory evidence of acute inflammation (e.g., highly elevated C-reactive protein, ferritin, D-dimer, or interleukin-6)" (Baum, 2020). Most patients present with a fever >100.4 °F, cardiac abnormalities (arrhythmias, elevated troponin levels, or left or right ventricular dysfunction), and gastrointestinal symptoms. Rare symptoms include dermatological manifestations or respiratory symptoms such as pleural effusion. Patients may have elevated laboratory markers of inflammation including CRP, ferritin, and markers of coagulopathy including D-dimer (Morris et al., 2020).

As SARS-CoV-2 has continuously mutated over the course of the pandemic, CDC has adjusted their categorizations of the numerous variants based on shared attributes that may require public action and on available information. CDC lists four variant classifications on their website: variants being monitored

(VBM), variants of interest (VOI), variants of concern (VOC), and variants of high consequence (VOHC). VBMs are described as "lineages with potential impact on available medical countermeasures based on analysis of genetic sequence data," "lineages that previously caused more severe disease or increased transmission but that are no longer detected", "lineage with an unusually large number of antigenic mutations AND presence in multiple countries with collection dates within 4 weeks", or "lineages previously designated as a VOI, VOC, or VOHC that are currently circulating at very low levels in the United States." As such, VBMs are "no longer circulating at sustained levels and no longer poses significant risk to public health in the United States" and VOIs and VOCs may be downgraded to this list when evidence suggests that they no longer pose significant risk to public health (CDC, 2024a). The list of possible attributes for variants of interest (VOIs) include the presence of "specific genetic markers that are predicted to affect transmission, diagnostics, therapeutics, or immune escape", and "evidence that it is the cause of an increased proportion of cases or unique outbreak clusters." In addition to including possible features of VOIs, VOCs are marked by a "increase in transmissibility," "more severe disease (for example, increased hospitalizations or deaths)," "significant reduction in neutralization by antibodies generated during previous infection or vaccination," and "reduced effectiveness of treatments or vaccines, or diagnostic detection failures." A VOHC "has clear evidence that prevention measures or medical countermeasures (MCMs) have significantly reduced effectiveness relative to previously circulating variants" (CDC, 2024a). Currently, all the variants being monitored by CDC fall in VBM status except for the Omicron strain (B.1.1.529 and descendant lineages), which is labeled a VOC.

The CDC indicates three vaccines as authorized and recommended to prevent COVID-19 in the US: Pfizer-BioNTech COVID-19 Vaccine, Bivalent; Moderna COVID-19 Vaccine, Bivalent; and Novavax COVID-19 Vaccine, Adjuvanted. The Pfizer-BioNTech and Moderna COVID-19 vaccines are mRNA vaccines, which instruct B and T lymphocytes to fight off that specific mRNA-encoded protein from COVID-19 in the event of future exposure. Novavax is a protein subunit vaccine that delivers pieces (spike proteins) of the virus that causes COVID-19, as well as an adjuvant that helps the immune system respond in the event of future exposure (CDC, 2024c).

Besides the viruses associated with SARS, MERS, and COVID-19, four other human coronaviruses (HCoVs) are currently known—229E, NL63, OC43, and HKU1. These four viruses are considered endemic to the human population, and they typically cause mild respiratory tract infections associated with the common cold; in fact, it is approximated that up to one-third of all "common colds" may be due to one of these four endemic human coronaviruses. These HCoVs can cause both upper and lower respiratory infections, but they typically result in relatively mild, or even asymptomatic, cases. In immunosuppressed individuals, including those with pre-existing pulmonary diseases, progression to acute respiratory failure can occur in some cases (Corman et al., 2019; Ludwig & Zarbock, 2020).

Nucleic Acid Testing for Human Coronavirus Infections

Coronaviruses are a family of enveloped, single-stranded positive-sense RNA viruses. During the initial phase of infection, the virus can be detected in respiratory specimen due to high concentrations of viral RNA **(Figure 1).** RT-PCR is a powerful molecular technique that synthesizes complimentary DNA (cDNA) from the initial RNA template and uses primers to manufacture multiple cDNA copies for analysis. RT-PCR, when used with appropriate primers targeting the SARS-CoV-2 RNA, is used to diagnose an acute infection. The CDC RT-PCR Diagnostic Panel detects SARS-CoV-2 virus in the upper and lower respiratory specimen. As depicted in **Figure 1**, the concentration of viral RNA decreases as the immune system fights the infection, and very low or undetectable viral RNA levels are typically present after an individual has recovered. Consequently, RT-PCR cannot be used to screen for a past infection. Another limitation to RT-PCR is that it does require specific instrumentation, and, therefore, is less amenable as a

rapid, point-of-care test. RT-PCR results of SARS-CoV-2 may fluctuate and become unstable over time, thus requiring other clinical diagnostic measures, such as computerized tomography imaging to supplement isolation, discharge, and any transfers during this epidemic (Li et al., 2020).

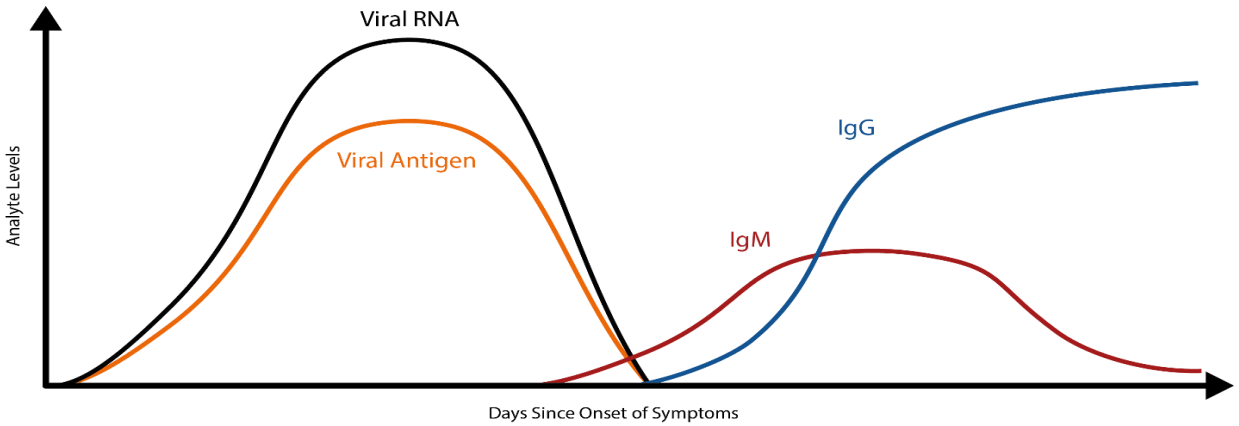


Figure 1: General time course of a viral infection, such as SARS-CoV-2. This is for illustrative purposes and should not be used as a primary reference or for diagnostic purposes. The original content can be found within the references (The Native Antigen Company, 2020).

Clinical Utility and Validity of Nucleic Acid Testing

Many studies have been performed to date to evaluate the analytical performance of RT-PCR. One study, using a high-throughput platform, for example, reported a limit of detection (LoD) of 689.3 copies/mL and 275.72 copies per reaction at 95% detection probability (Pfefferle et al., 2020). The WHO diagnostic RT-PCR test utilizes two genes--the *E* gene as the molecular target (where the limit is 3.9 copies per reaction) and the *RdRp* gene as the molecular target (limit of 3.6 copies per reaction) (Lippi et al., 2020). One recent study reported possible *in vitro* cross-reactivity between the RdRp-based method used predominantly in European labs with SARS-CoV in cell culture (Chan et al., 2020). SARS-CoV is the coronavirus that caused the initial SARS (Severe Acute Respiratory Syndrome) outbreak in 2003 (WHO, 2024b). The likelihood of either a co-infection of SARS-CoV and SARS-CoV-2 or a concurrent outbreak of both viruses is small. The CDC diagnostic panel test does not target the *RdRp* gene; it consists of two primer/probe sets of the *N* gene and one primer/probe set for human RNase P gene (*RP*) as the control. The CDC diagnostic panel has a reported limit of 1.0 – 3.2 copies/µL (Lippi et al., 2020). Reports of initial negative RT-PCR results in individuals who later develop symptomatic COVID-19 have been published, but this may occur if the sample was not properly collected or if it was taken from the patient early in the infection during the initial incubation period of SARS-CoV-2, which is approximately six days (interquartile range [IQR], 2 – 11 days) (Backer et al., 2020; Lippi et al., 2020). Consequently, it is important to remember that "Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information" (LabCorp, 2022a, 2022b).

To compare and analyze the diagnostic efficacy of two RT-PCR test kits for detection of SARS-CoV-2, Lu et al. (2020) studied throat swab samples from 18 hospitalized patients with a clinical COVID-19 diagnosis and 100 hospitalized patients without COVID-19 diagnosis. Two different RT-PCR tests from Sansure Biotech Inc (SansureBiotech, 2022) and Shanghai BioGerm Biotechnology Co., Ltd (BioGerm, 2024) were used. **Table 2** (Lu et al., 2020) shows that the detection efficacy of the BioGerm PCR kit was higher than

that of the Sansure PCR kit. These two kits had the same specificity and positive predictive value, but the sensitivity of the Sansure PCR kit was 83.3%, whereas the sensitivity of the BioGerm PCR kit was 94.4%. For the Sansure PCR kit, three of the 18 samples were false-negative results, and for the BioGerm PCR kit, one of the 18 samples was a false-negative result. No false-positive results were detected in these tests. The author suggests that "these findings provide important information for the ongoing optimization of viral detection assays following the emergence of COVID-19" (Lu et al., 2020).

	COVID-19 samples (n = 18)		**None-COVID-19 samples (n = 100)**		**Sensitivity (95%CI)**	**Specificity (95%CI)**	**PPV (95%CI)**	**NPV (95%CI)**	**Kappa (95%CI)**
Test kits	**Positive**	**Negative**	**Positive**	**Negative**					
Sansure	15	3	0	100	0.833(0.577-0.956)	1.000(0.954-1.000)	1.000(0.747-1.000)	0.971(0.911-0.992)	0.894(0.726-1.000)
BioGerm	17	1	0	100	0.944(0.706-0.997)	1.000(0.954-1.000)	1.000(0.771-1.000)	0.990(0.938-0.999)	0.966(0.880-1.000)

Table 2. *Diagnosis efficacy of Sansure and BioGerm test kits for SARS-CoV-2 nucleic acid detection*

In a case series study of multisystem inflammatory syndrome in adults (MIS-A) associated with SARS-CoV-2 infection, 16 patients ranging from 21 to 50 years old were enrolled and tested with PCR assay. Ten out of 16 patients had positive SARS-CoV-2 PCR test results at the time of admission. Two patients had positive SARS-CoV-2 PCR test results 14 and 37 days before admission and negative PCR results at the time of admission. Three patients had positive SARS-CoV-2 PCR test results 25–41 days before admission and continued positive PCR test results at the time of admission. "Given the high proportion of MIS-C patients with negative PCR testing, clinical guidelines recommend the use of both antibody and viral testing to assist with diagnosis" (Morris et al., 2020).

Li et al. (2021) conducted a cross-sectional analysis on 30 patients with COVID-19 diagnoses to compare the sensitivity of SARS-CoV-2 testing in anterior nasal vestibular swabs versus oropharyngeal swabs. After specimen collection, RT-PCR assays were used to test them for SARS-CoV-2. They found that 56.7% of the patients tested positive using oropharyngeal specimen, whereas 66.7% of patients tested positive with the nasal swab specimens. Ultimately, there is "adequate sensitivity" to use the less invasive anterior nasal vestibular swabs to detect COVID-19 infection confirmed by RT-PCR (Li et al., 2021).

Yau et al. (2021) evaluated the clinical utility of a rapid "on-demand" PCR-based testing service in an acute hospital setting. To increase hospital efficiency starting from July 2020, the researchers focused on moving patients quickly to isolation rooms and minimize potential risk of transmission in crowded areas.

From their study, it was found that the "daily/monthly PCR positive test numbers approximately followed the local and national UK trend in COVID-19 case numbers, with the daily case numbers being reflective of the Nov and Dec 2020 surges." It ultimately helped to reduce "unnecessary 'length-of-stay' in a busy acute respiratory ward." Patients were able to be rapidly separated based on COVID-19 positive diagnosis and the system in place reduced exposure and nosocomial transmission (Yau et al., 2021).

Dighe et al. (2022) studied a lateral flow strip-based RNA extraction and amplification-free nucleic acid test (NAT) for rapid diagnosis of COVID-19 at point of care which takes no longer than 30 minutes. This test uses highly specific 6-carboxyfluorescein (6-FAM) and biotin labeled antisense oligonucleotides (ASOs) as probes those are designed to target the N-gene sequence of COVID-19. This study evaluated 60 samples using the lateral flow assay and results were compared with the FDA approved TaqPath RT-PCR kit. According to the results, the assay obtained almost 99.99% accuracy and specificity. The authors conclude that this new LFA method could be "expanded beyond COVID-19 detection, simply by altering its targeting antisense oligonucleotides, to become a global health technology that contributes to providing low-cost diagnostics" (Dighe et al., 2022).

Mawhorter et al. (2022) investigated the impact and cost of a routine pre-operative COVID-19 PCR testing algorithm for asymptomatic patients before elective surgery at a rural academic institution per recommendations by the American College of Surgeons. From 7579 pre-procedural tests that were completed since May 2020 using the protocol, the study yielded 31 (0.41%) positive results in asymptomatic patients. With these positive results, there were impacts on both the cost and delay of the procedure. The results showed that "20 procedures (62.5%) were delayed an average of 49 days, 8 were not performed, and 3 proceeded without delay," with a prolonged delay for the three urological procedures of 59 days. They also identified that the number needed to test for one positive result was 244, with $11,573 as cost for each positive result. This analysis found that the hospital was able to be more cost-effective (each test was $34-54) with a standardized testing algorithm prior to procedure performance (Mawhorter et al., 2022).

Host Antibody Testing

The COVID-19 illness begins with an initial infection by SARS-CoV-2. Viral invasion stimulates the host immune response to produce immunoglobulins, such as IgM, IgA, and IgG, that can target the invading virus. However, there is a delay between the time of initial infection and the production of immunoglobulins **(Figure 1)** (The Native Antigen Company, 2020). Typically, several days after the initial onset of symptoms, the first IgM immunoglobulins are produced to combat the viral infection. IgA (not shown in Figure 1), immunoglobulins secreted to protect predominantly the mucosal linings of the gastrointestinal, respiratory, and genitourinary tracts (Woof & Kerr, 2006), typically have a half-life of four to six days (Morell et al., 1973). Finally, IgG, the long-term immunoglobulins found within body fluids that fight bacterial and viral infections, are produced and IgM production wanes. Some limited studies have indicated that some individuals may initially produce IgM and IgG antibodies concurrently, but additional research is needed (Padoan et al., 2020).

Serological host antibody tests can detect the presence of IgM and IgG antibodies that an individual has developed in response to an infection—in this case, a SARS-CoV-2 viral infection. The test may report total antibodies present, meaning either it does not distinguish between IgG and IgM or that it is reporting the sum of IgG and IgM. This is sometimes referred to as "total antibody testing." On the other hand, the test may be specific for one antibody, such as IgG or IgM, or the test may claim to accurately distinguish between the antibodies.

Another type of antibody testing is "neutralizing" antibody detection, as opposed to "binding" antibody detection described above. This process involves incubating serum with a live version of the virus. The analytes of interest are the antibodies that have the capability to prevent infection by the virus (i.e., neutralization). Identification of these antibodies may contain useful clinical information and are often reported in an aggregate titer, as opposed to specifying each individual antibody (Espejo et al., 2020).

Clinical Utility and Validity of Host Antibody Testing

Antibody testing has many potential uses. Ideally, the use of an accurate, reliable antibody test could possibly show whether someone has previously been exposed to the virus. This could indicate possible immunity in an individual. Please note that ***the antibody test is not used as a diagnostic test, meaning it should not be used to diagnose an acute infection***. Within the FDA policy for diagnostic testing for COVID-19, issued on November 15, 2021 they state, "Results from antibody testing should not be used to diagnose or exclude SARS-CoV-2 infection" (FDA, 2023b).

Since SARS-CoV-2 is a new, emerging virus, it is not known for certain how long it takes for the seroconversion to occur or when antibodies start to appear in the blood at high enough concentrations for accurate testing results. A recent study published in *Clinical Infectious Diseases* reports an average of seroconversion time for IgM and IgG at 12 and 14 days, respectively (Zhao et al., 2020). A small study (n=34 patients) reports the presence of IgG for at least seven weeks (the duration of the study) (Xiao et al., 2020). Another study, however, reports that IgM testing has similar, if not better positive detection rate than PCR 5.5 days after initial onset of symptoms; however, the total window of antibody detection for IgM was only five days long (Guo et al., 2020) **(See Figure 1)**. If the patient was not tested during the detection window, then the individual would not necessarily have a "positive" result for IgM. The authors also report the detection of IgA antibodies (median onset at five days after initial symptoms [IQR three – six days]), and 92.7% of total samples report a positive result for IgA. This same study also reports that IgG detection occurs, on average, fourteen days after initial onset of symptoms (Guo et al., 2020). Another study reports that IgA-based ELISA testing has higher sensitivity than IgG-based ELISA testing, but the IgG-based ELISA testing has higher specificity. The authors recommend IgG-based testing over the IgA-based testing in immunosurveillance studies since IgG has a longer biological half-life (Okba et al., 2020). At least one published study to date has reported that as many as 6.9% of individuals who previously had tested positive with RT-PCR results did not show the presence of antibodies for the length of the study (at least 40 days after the initial onset of symptoms) (Zhao et al., 2020).

Ideally, any rapid diagnostic test for the outpatient setting must be accurate and reliable. Current research indicates that the diagnostic window for IgA and IgM is very limited. Some data indicate that host antibody testing can also yield inaccuracies. Also, for IgG testing, the significance of positive results is questionable at the current time. A positive result could indicate a previous infection, assuming the test did not cross-react with any other IgG the host produced in response to one of the four coronaviruses known to cause the common cold in humans, for example. It is not currently known, however, if the presence of IgG antibodies indicates immunity (or degree thereof) of the host against SARS-CoV-2. The duration of any conferred immunity, or the level of IgG antibodies required to effectively acquire such immunity, are also unknown. Additional research is needed and encouraged.

Lisboa Bastos et al. (2020) performed a meta-analysis to investigate the diagnostic accuracy of serological testing for COVID-19. The authors aimed to identify studies where serological testing was compared to the "reference standard of viral culture or reverse transcriptase polymerase chain reaction." The authors identified a total of 40 studies for inclusion in the study. The pooled sensitivity of enzyme linked immunosorbent assays (ELISAs) measuring IgG or IgM to be 84.3% (with a 95% confidence

interval [CI] of 75.6%-90.9%). For lateral flow immunoassays (LFIAs), the pooled sensitivity was found to be 66% (95% CI: 49.3%-79.3%), and for chemiluminescent immunoassays (CLIAs), the pooled sensitivity was found to be 97.8% (95% CI: 46.2%-100%). Pooled specificities ranged from 96.6%-99.7%. Sensitivity was also found to be higher at least three weeks from symptom onset (69.9% to 98.9%) compared to within the first week (13.4% to 50.3%) Of the samples used to calculate specificity, 83% were "from populations tested before the epidemic or not suspected of having COVID-19." The authors performed 49 bias risk assessments (one for methodology and one for patient selection) and identified 48 with a "high risk of patient selection bias" and 36 with "high or unclear risk of bias from performance or interpretation of the serological test." The authors also noted that only four of the forty studies including outpatients and only two studies evaluated point-of-care testing. The authors concluded that "currently, available evidence does not support the continued use of existing point-of-care serological tests" but acknowledged that "higher quality clinical studies assessing the diagnostic accuracy of serological tests for covid-19 are urgently needed" (Lisboa Bastos et al., 2020).

Kontou et al. (2020) performed a meta-analysis investigating the use of antibody tests in detecting SARS-CoV-2. The authors focused on IgG and IgM tests based on enzyme-linked immunosorbent assays (ELISA), chemiluminescence enzyme immunoassays (CLIA), fluorescence immunoassays (FIA), and lateral flow immunoassays (LFIA). A total of 38 studies encompassing 7848 individuals (3522 COVID-19 cases, 4326 healthy controls) were included. Of the 38 studies, 21 included data for both COVID-19 cases and controls. Fourteen studies using ELISA were included, and the authors found that IgG and IgM perform "similarly" individually, but in combination, resulted in a sensitivity of 0.935. Thirteen studies using CLIA resulted in an IgG sensitivity of 0.944, an IgM sensitivity of 0.810, and a combined IgG/IgM sensitivity of 0.910. The specificities ranged from 0.954 to 0.984. Thirteen studies used LFIA and found the IgG and IgM sensitivities to range from 0.53-0.66. Combining IgG and IgM resulted in sensitivities of 0.78-0.83. The authors also attempted to analyze FIA-based studies but were unable to due to the paucity of studies (three identified). The authors concluded that ELISA- and CLIA-based testing performed better sensitivity-wise and that LFIA studies are "more attractive for large seroprevalence studies but show lower sensitivity" (Kontou et al., 2020).

Ko et al. (2020) investigated the differences in neutralizing antibody production between asymptomatic and "mild" symptomatic COVID-19 patients, compared to pneumonic COVID-19 patients. A total of 70 patients (15 asymptomatic, 49 mild symptomatic, and six pneumonic) were included. A microneutralization assay was performed, along with a FIA and ELISA. Neutralizing antibody production was observed in all the pneumonic patients, 93.9% of the mildly symptomatic patients, and 80% of the asymptomatic patients. Further, the entire pneumonic group showed "high" titer (defined as ≥1:80), while 36.7% of the mild group and 20% of the asymptomatic group showed high titer. Both the FIA (for IgG) and ELISA detected anti SARS-CoV-2 at a high sensitivity (98.8% and 97.6% respectively). The authors concluded that "Most asymptomatic and mild COVID-19 patients produced the neutralizing antibody, although the titers were lower than pneumonia patients" (Ko et al., 2020).

Wu et al. (2020) investigated the association between levels of neutralizing antibodies (NAbs) and clinical characteristics in recovered COVID-19 patients. A total of 175 patients with "mild" symptoms of COVID-19 were included. The authors found that NAbs were detected in patients starting in days 4-6 and reached peak levels in days 10-15. NAbs were also found not to cross-react with SARS-associated CoV, but correlated with "spike-binding antibodies targeting S1, receptor binding domain, and S2 regions. The authors also noted that NAbs titers were "significantly" higher in 56 "older" patients (1537 [IQR, 877-2427]) and 63 "middle-aged" patients (1291 [IRQ, 504-2126]) compared to 56 "younger patients" (459 [IQR, 225-998]). The authors concluded that "…NAb titers to SARS-CoV-2 appeared to

vary substantially. Further research is needed to understand the clinical implications of differing NAb titers for protection against future infection" (Wu et al., 2020).

Kweon et al. (2020) collected 97 samples from patients with COVID-19 to analyze the serologic profiles and time kinetics of IgG and IgM against SARS-CoV-2 using the AFIAS COVID-19 Ab (BodiTechMed, 2024) and the EDI™ Novel Coronavirus COVID-19 ELISA Kit (EpitopeDiagnostics, 2024). The AFIAS assay uses recombinant nucleocapsid protein as an antigen to determine IgG and IgM antibodies against SARS-CoV-2 within 20 minutes from whole blood, serum, or plasma. The EDI™ ELISA Kit uses the microplate-based enzyme immunoassay technique to detect antibodies by measuring the optical densities (ODs) of each well of immunocomplexes. To determine the kinetics of antibodies, studies were performed at different past symptom onset (PSO) periods and to determine diagnostic accuracy of serologic assays, diagnostic sensitivity and specificities were calculated by PSO of ≤14 days and >14 days. Kinetic studies showed that "with both assays, IgM and IgG rapidly increased after seven days post symptom onset (PSO). IgM antibody levels reached a peak at 15–35 d PSO and gradually decreased. IgG levels gradually increased and remained at similar levels after 22–35 d" (Kweon et al., 2020). The diagnostic accuracy of both serologic assays also differed based on PSO. "The sensitivity of IgG samples from ≤14 d PSO was as low as 35.7%~57.1%, but it sharply increased for >14 d PSO to 88.2%~94.1%. This means that almost all patients with COVID-19 showed seroconversion after 14 d PSO, and IgG seronegative subjects in this period are considered less likely to be infected with SARS-CoV-2. In addition, both assays showed 94.2~96.4% of IgG specificities and increased IgG titers in COVID-19 patients were maintained. Thus, IgG serologic assays can be useful for ruling out SARS-CoV-2 infection after 14 d PSO, detecting past infection, and epidemiologic surveys" (Kweon et al., 2020). For IgM, the sensitivities were "as low as 21.4% (same in both assays) in the samples collected ≤14 d PSO and 41.2%~52.9% in samples >14 d PSO. These findings indicated that in patients infected with SARS-CoV-2, IgM seroconversion may not develop or might not be detected until the middle or late stages of infection. In other words, SARS-CoV-2 infection may be missed based on IgM seropositivity; thus, IgM tests must not be solely used in COVID-19 diagnosis and should be used only as a supportive tool in addition to molecular tests" (Kweon et al., 2020). In addition, IgM titers in COVID-19 patients showed a significant reduction after 35 d PSO; therefore, their utility in detecting past infection is limited. The author concludes that "testing for antibodies against SARS-CoV-2, especially IgG, has the potential for ruling out SARS-CoV-2 infection after 14 d PSO, detecting past infection, and epidemiologic surveys" (Kweon et al., 2020).

Caturegli et al. (2020) performed a case-control study to determine the clinical utility and validity of using SARS-CoV-2 antibodies, which were serum IgG and IgA antibodies formed against the SARS-CoV-2 spike protein detected by enzyme-linked immunosorbent assay (ELISA). When assays were formed 14 days or later after symptom onset, the researchers found that the sensitivity was 0.976 (95% CI, 0.928 to 0.995) and specificity was 0.988 (95% CI, 0.974 to 0.995), but the sensitivity decreased at earlier time points. Antibodies "predicted the odds of developing acute respiratory distress syndrome, which increased by 62% (CI, 48% to 81%; $P < 0.001$) for every 2-fold increase in IgG." This demonstrates the linkage of antibodies used to measure clinical severity and for those who tested negative by NAAT but remained potentially COVID-positive.

In a household cohort study, Churiwal et al. (2021) assessed the utility of a rapid point of care test for COVID-19 antibodies by comparing the performance of BioMedomics COVID-19 IgM/IgG Rapid Antibody Test against an ELISA. The test was performed on 303 patients at study enrollment and four weeks later. According to the results, sensitivity was lower early in infection and those who never developed symptoms (74% sensitivity). Only two were detected among 499 tests early in infection due to false-positive IgM bands. When measured four weeks later after the onset of symptoms, it demonstrated

robust sensitivity (90%) and complete specificity (100%). The authors conclude that "When used appropriately, rapid antibody tests offer a convenient way to detect symptomatic infections during convalescence" (Churiwal et al., 2021).

Fox et al. (2022) performed a meta-analysis to assess the accuracy of antibody tests. The analysis covered 178 studies with a total of 64,688 samples taken from 25,724 people with confirmed SARS-CoV-2. All the studies were conducted before the introduction of the SARS-CoV-2 vaccines to ensure the responses were due to naturally acquired antibodies. The average sensitivity for either IgG or IgG combined with IgM was 41.1% one week after symptom onset, 74.9% two weeks after symptom onset, and 88.0% three weeks after symptom onset. The average sensitivity during the convalescent phase of infection, up to 100 days since symptom onset, was 89.8% for IgG, 92.9% for IgG or IgM combined, and 94.3% for total antibodies. The average sensitivities for IgM alone "followed a similar pattern but were of a lower test accuracy in every time slot." The authors conclude that antibody tests "could be a useful diagnostic tool" but note that "antibody tests have an increasing likelihood of detecting an immune response to infection as time since onset of infection progresses and have demonstrated adequate performance for detection of prior infection for sero-epidemiological purposes" and "the applicability of results for detection of vaccination-induced antibodies is uncertain" (Fox et al., 2022).

Antigen Testing

Another possible diagnostic testing methodology is antigen detection testing, which relies upon the direct detection of parts of the virus called "antigens"—in this instance, proteins located on the outside of SARS-CoV-2, such as the spike protein (S) or nucleocapsid protein, that can cause an immune response in an individual. What makes this method of testing distinct from antibody testing is that antigen testing directly measures the presence of the virus in a person whereas antibody testing is measuring the patient's response to an infection. These antigen detection tests can be deployed as rapid antigen tests that decrease the turnaround time for results but usually lack specificity (Loeffelholz & Tang, 2020).

On May 8, 2020, the FDA issued the first EUA for antigen testing for COVID-19 to the Quidel Corporation for their Sofia®2 SARS Antigen FIA lateral flow immunofluorescent sandwich assay for the qualitative detection of the nucleocapsid (N) protein antigen of SARS-CoV-2 for use in individuals suspected of COVID-19 by their healthcare provider (Quidel Corporation, 2020). This test has been approved as a point-of-care (POC) test (FDA, 2024c). This test functions by detecting the N protein of either the SARS-CoV or SARS-CoV-2 virus from an upper respiratory sample (either a nasal swab or nasopharyngeal swab). First, the sample is placed in a reagent tube so that any virus, if present, is broken apart to allow for the N proteins to be exposed. The sample then travels from the sample well, down a test strip—where the term "lateral flow" is derived—where the proprietary reagents will recognize any N proteins and trap them in place on the strip. The test requires at least 15 minutes to develop prior to analysis. The strip can then be read by the Sofia®2 system that measures the fluorescent signal from the proprietary reagents. The Sofia®2 system allows the user to have two different modes for analysis—"Walk Away" and "Read Now." For the "Walk Away" mode, the user will insert the test cassette strip into the system, and the results will be displayed in 15 minutes because the test will be developed while in the instrument. In "Read Now" mode, the user must have already allowed at least 15 minutes for the test to develop prior to inserting it into the instrument. Then, the Sofia®2 system will display the result within one minute (Quidel Corporation, 2020). On August 20, 2020, Quidel reported that the Sofia test's labeling had been amended to include "either nasal or nasopharyngeal swabs" thereby allowing Quidel a second corresponding kit configuration (BioSpace, 2020).

On July 2, 2020, a second antigen test (BD Veritor System for Rapid Detection of SARS-CoV-2) from Becton, Dickinson, and Company was issued an EUA. This test is described as "a chromatographic digital immunoassay intended for the direct and qualitative detection of SARS-CoV-2 nucleocapsid antigens in nasal swabs from individuals who are suspected of COVID-19 by their healthcare provider within the first five days of the onset of symptoms." The test is authorized for use in POC settings. The test's mechanism of action is as follows: if there are any antigens in the sample (in this case, the nucleocapsid of the virus), they will bind to antibodies conjugated to detector particles in the test strip. The new "conjugates" migrate to the "reaction area" and are captured by another line of antibodies. The test reads positive when the conjugate is found at both "Control" and "Test" positions on the device. BD Veritor reported the following values for the test (in comparison to RT-PCR): 84% positive predictive agreement, 100% negative predictive agreement, 98% overall percent agreement, 100% positive predictive value, and 97.5% negative predictive value. No cross-reactivity was reported (BD Veritor, 2020).

On August 18, 2020, a third antigen test (LumiraDx SARS-CoV-2 Ag Test from LumiraDx UK Ltd.) was issued an EUA. The test is described as "a single use fluorescence immunoassay device designed to detect the presence of the nucleocapsid protein antigen directly from SARS-CoV-2 in nasal swab specimens, without transport media." The mechanism of action is as follows: when a droplet of the specimen is added to the "Test Strip," pre-made reagents on the strip react with any antigen in the specimen. The amount of fluorescence created is proportional to the amount of antigen detected. LumiraDx reported a limit of detection of 32 TCID50/mL [tissue culture infectious dose], as well as a 97.6% positive percent agreement, 96.6% negative percent agreement, 93.1% positive predictive value, 98.8% negative predictive value, and 96.9% overall percent agreement (based on 257 total samples) (LumiraDx, 2020).

As of April 20, 2022, 50 antigen tests have Emergency Use Authorization (EUA) by the U.S. Food and Drug Administration (FDA) (FDA, 2023a). These testing methods include (among others): Bulk Acoustic Wave (BAW) Biosensors, Chemiluminescence Immunoassays, Chromatographic Digital Immunoassays, Digital Lateral Flow, Magnetic Force-assisted Electrochemical Sandwich Immunoassay (MESIA), Microfluidic Immunofluorescence Assay, and Paramagnetic Microbead-based Immunoassay (FDA, 2023a).

Clinical Utility and Validity of Antigen Testing

To address the clinical performance, two primary studies were performed. Both studies only used frozen samples. The first study used 143 samples with 80% PPA or Positive Percent Agreement (47/59 of positive samples tested "positive"). They report 100% NPA or Negative Percent Agreement—all 84 negative samples tested "negative." The second study used a total of 48 samples. Again, 80% of the positive samples tested "positive"; however, only a total of five positive samples were included within this second study. The remaining 43 samples were all negative samples. This study reports a sensitivity of 80.0%, but a 95% confidence interval range of 37.6% - 96.4%. A third supportive study was also performed. In this study, thirty swabs were taken. Twenty of these swabs were spiked with one lower concentration of the virus while the remaining ten swabs were spiked with a higher concentration of the virus. Then, all 30 swabs were tested and compared to 47 control ("unspiked") samples. In this study, none of the "unspiked" control samples tested "positive" while all 30 of the "spiked" samples, regardless of the concentration, tested positive. Quidel also tested the LoD of the Sofia®2 SARS Antigen FIA test. LoD is typically measured by determining the $TCID_{50}$ (median tissue culture infective dose). The $TCID_{50}$ is the amount where 50% of the cells within a sample are infected (Wulff et al., 2012). For the Sofia®2 SARS Antigen FIA test, the LoD for a direct swab sample has a $TCID_{50}$ of 113 mL whereas it is 850 mL if the initial sample is from a swab sample that has been diluted into three mL of reagent. Finally, Quidel

also checked this antigen test for possible cross-reactivity with several microorganisms and other viruses. It shows no cross-reactivity with any of the microorganisms or viruses tests other than SARS-CoV. Of note, it does not cross-react with human coronavirus 229e, OC43, NL63, or MERS-CoV (heat-inactivated); however, they did not check for possible cross-reactivity with the other known human coronavirus (HKU1) due to a lack of availability at this time. This is noteworthy since this coronavirus is associated with the common cold. Limitations of the Sofia®2 SARS Antigen FIA test includes the following:

- This test must be performed using the Sofia®2 system, and the test must be performed accurately following the test procedure. Failure to do so can adversely affect the performance of the test and may invalidate the results.
- A positive test cannot distinguish between a SARS-CoV or a SARS-CoV-2 infection. SARS-CoV is the virus that caused the SARS outbreak of 2003. It should be noted that there is no current outbreak of SARS.
- This test also does not distinguish between "live" (viable) virus and non-viable virus. Consequently, the test results do not necessarily correlate with viral culture results performed on the same sample.
- This test is only for the qualitative use on a sample from either a nasal swab or a nasopharyngeal swab. It has not been approved for use, at this time, on any other sample, such as saliva.
- Negative test results can occur if the viral level is below the lower limit of the test. All negative results "should be treated as presumptive and confirmed with an FDA authorized molecular assay, if necessary, for clinical management, including infection control" (Quidel Corporation, 2020).
- Positive test results do not rule out coinfections, and negative results do not "rule in" other non-SARS viral or bacterial infections.
- The clinical performance assays submitted for FDA approval were performed using frozen samples; the test may have a different performance when used with a fresh sample (such as in a point-of-care setting).
- "If the differentiation of specific SARS viruses and strains is needed, additional testing, in consultation with state or local public health departments is required" (Quidel Corporation, 2020).
- As previously noted, the company did not check this test (as of publication date) for cross-reactivity with human coronavirus HKU1 due to a lack of availability of that strain. This is notable since this virus is associated with upper respiratory conditions such as the common cold.

One multi-center study, currently a preprint at the time of publication, reports the development of another rapid antigen detection test (RADT) that screens for SARS-CoV-2 by targeting the nucleocapsid protein. This test, when using a nasopharyngeal swab sample, reports a 100% positive agreement with RT-PCR testing. They also report 73.6% positive agreement when using a urine sample (Diao et al., 2020). This study is yet to be published in a peer-reviewed journal, and the test is not FDA approved as of May 18, 2020. Another study published recently in *ACS Nano* reports on the development of a RADT using field-effect transistor (FET)-based biosensing where a graphene sheet for the FET is coated with a specific antibody against the SARS-CoV-2 spike protein. This method can detect the protein in concentrations as low as one fg/mL in buffer and has an LOD of 242 copies/mL for a clinical sample (versus 16/mL for a culture medium) (Seo et al., 2020). To date, the WHO states that "Ag-RDTs could play a significant role in guiding patient management, public health decision making and in surveillance of COVID-19. Currently, there is insufficient evidence on performance and operational use to recommend specific commercial products" (WHO, 2021a).

Scohy et al. (2020) evaluated the Coris COVID-19 Ag [Antigen] Respi-Strip test in comparison to RT-PCR. The authors tested 148 nasopharyngeal swabs, with 106 testing positive by RT-PCR. The rapid antigen

test detected 32 of these 106 positive results, for a sensitivity of 30.2%. All samples deemed positive by the antigen test were also deemed positive by RT-PCR. The authors noted that higher viral loads were associated with better detection by antigen tests but concluded that "the overall poor sensitivity of the COVID-19 Ag Respi-Strip does not allow using it alone as the frontline testing for COVID-19 diagnosis" (Scohy et al., 2020).

Mak et al. (2020) evaluated the BIOCREDIT COVID-19 Ag test in comparison to RT-PCR. The BIOCREDIT test's limit of detection (LOD) was compared to RT-PCR and viral culture, and a total of 368 samples from confirmed COVID-19 cases were included. A sample volume of 100 µL was used. The authors found the LOD of BIOCREDIT to be 1000-fold less sensitive than viral culture (BIOCREDIT LOD: 10^{-2}, viral culture: 10^{-5}). RT-PCR's LOD was measured to be 10^{-7}. Further, BIOCREDIT detected between 11.1% and 45.7% of RT-PCR positive patients from COVID-19 patients. The authors concluded that "This study demonstrated that the RAD test serves only as adjunct to RT-PCR test because of potential for false-negative results" (Mak et al., 2020).

Lambert-Niclot et al. (2020) analyzed the COVID-19 Ag Respi-Strip test and compared its accuracy to RT-PCR. A total of 138 nasopharyngeal samples were included, with 94 testing positive by RT-PCR. The Respi-Strip test identified 47 of 94 positive specimens for a sensitivity of 50%, although the specificity was 100% for both tests. The authors also noted that the control lines were "barely" visible for 17 tests (nine positive and eight negative). The authors acknowledged that due to the low prevalence in France (the country in which this study was performed), prospective studies should be undertaken(Lambert-Niclot et al., 2020).

Hirotsu et al. (2020) evaluated a new antigen test (LUMIPULSE) which is based on chemiluminescence enzyme immunoassay. A total of 313 nasopharyngeal swabs were included (82 serial samples from seven COVID patients, 231 individual samples from four COVID patients and 215 healthy controls). These samples were tested by both LUMIPULSE and RT-PCR. Compared to RT-PCR, LUMIPULSE demonstrated a 91.4% overall agreement rate (286/313), with a 55.2% sensitivity and 99.6% specificity. At >100 viral copies, LUMIPULSE agreed perfectly with RT-PCR, and at 10-100 viral copies, there was an 85% concordance rate (with concordance declining at lower viral loads). The authors concluded that "the LUMIPULSE antigen test can rapidly identify SARS-CoV-2-infected individuals with moderate to high viral loads and may be helpful for monitoring viral clearance in hospitalized patients" (Hirotsu et al., 2020).

Villaverde et al. (2021) conducted a multicenter study to compare the diagnostic accuracy of the Panbio coronavirus disease 2019 Antigen Rapid Test of nasopharyngeal samples in pediatric patients with COVID-19 symptoms ≤5 days. They demonstrated "limited accuracy in nasopharyngeal antigen testing: overall sensitivity was 45.4%, and 99.8% of specificity, positive-predictive value was 92.5%," with moderate concordance between the RT-PCR and antigen test. They noted that a high proportion of false-negative results from the antigen tests (54.5%) may have public health implications in unknown spreading of the virus. But because this test has a good positive likelihood ratio, and is cheap, rapid, and widely distributed, it may be used as a first screening test in a pandemic situation, though its value as a diagnostic tool is questionable due to the low sensitivity and negative likelihood ratio.

Peacock et al. (2022) studied the clinical utility of the BinaxNOW antigen test by Abbott Diagnostics, a lateral flow immunochromatographic point-of-care test which provides results in 15 minutes from a nasal swab. BinaxNOW was performed on 735 samples and results were compared to PCR. In total, 623 of 735 (84.8%) had symptoms and 460 of 623 patients (62.6%) had symptoms for less than seven days. Positive tests occurred in 173 (23.5%) for the PCR and 141 (19.2%) with the BinaxNOW test. Those with

symptoms for more than two weeks had a positive test rate half of those with earlier onset. "In patients with symptoms ≤7 days, the sensitivity, specificity, and negative and positive predictive values for the BinaxNOW test were 84.6%, 98.5%, 94.9%, and 95.2%, respectively" (Peacock et al., 2022). The authors conclude that BinaxNOW has good sensitivity and specificity and is recommended for patients with symptoms up to two weeks (Peacock et al., 2022).

Panel Testing

Multiple laboratories have developed panels to screen for possible microorganism infections from a single sample. For example, multiplex PCR can simultaneously detect multiple pathogens rather than sequentially testing for each individual pathogen. Such testing can be advantageous when different pathogens may manifest with similar clinical presentation; however, this testing can be costly and can also result in false-negatives if preferential amplification of one target over another occurs. As of May 4, 2022, the BioFire® Respiratory Panel 2.1 (RP2.1), the QIAstat-Dx® Respiratory SARS-CoV-2 Panel, ePlex Respiratory Pathogen Panel 2, cobas SARS-CoV-2 & Influenza A/B, Xpert Xpress SARS-CoV-2/Flu/RSV, Quest Diagnostics RC COVID-19 +Flu RT-PCR, Sofia 2 Flu + SARS Antigen FIA, and the Influenza SARS-CoV-2 (Flu SC2) Multiplex Assay from the CDC received an EUA from the FDA for testing for COVID-19 (FDA, 2024c). The BioFire® Respiratory Panel 2.1, the QIAstat-Dx® Respiratory SARS-CoV-2 Panel, and ePlex Respiratory Pathogen Panel 2 use multiplex nucleic acid testing from a nasopharyngeal swab to detect and differentiate microorganisms listed in Table 1 (BioFire, 2020; GenMark Diagnostics, 2024; Qiagen GmbH, 2021), whereas the CDC Multiplex detects and differentiates influenzas A and B from SARS-CoV-2 (FDA, 2021c).

Table 1: Respiratory Pathogen Panel Testing Containing SARS-CoV-2

BioFire® Respiratory Panel 2.1	QIAstat-Dx® Respiratory SARS-CoV-2 Panel	ePlex Respiratory Pathogen Panel 2

• **Adenovirus**
• **HCoV 229E**
• **HCoV HKU1**
• **HCoVNL63**
• **HCoV OC43**
• **SARS-CoV-2**
• **Human Metapneumovirus**
• **Human Rhinovirus/Enterovirus**
• **Influenza A**
  ○ **Subtype H1**
  ○ **Subtype H3**
  ○ **Subtype H1-2009**
• **Influenza B**
• **Parainfluenza Virus 1**
• **Parainfluenza Virus 2**
• **Parainfluenza Virus 3**
• **Parainfluenza Virus 4**
• **Respiratory Syncytial Virus**
• ***Bordetella parapertussis***
	• Adenovirus
	• HCoV 229E
	• HCoV HKU1
	• HCoVNL63
	• HCoV OC43
	• SARS-CoV-2
	• Human Metapneumovirus A+B
	• Influenza A
	  ○ Subtype H1
	  ○ Subtype H3
	  ○ Subtype H1N1/pdm09
	• Influenza B
	• Parainfluenza Virus 1
	• Parainfluenza Virus 2
	• Parainfluenza Virus 3
	• Parainfluenza Virus 4
	• Rhinovirus/Enterovirus
	• Respiratory Syncytial Virus A+B
	• *Bordetella pertussis*
		• Adenovirus
		• HCoV 229E
		• HCoV HKU1
		• HCoVNL63
		• HCoV OC43
		• SARS-CoV-2
		• Human Metapneumovirus A+B
		• Influenza A
		  ○ Subtype H1
		  ○ Subtype H3
		  ○ Subtype H1-2009
		• Influenza B
		• Parainfluenza Virus 1
		• Parainfluenza Virus 2
		• Parainfluenza Virus 3
		• Parainfluenza Virus 4
		• Rhinovirus/Enterovirus
		• Respiratory Syncytial Virus A+B
		• *Chlamydia pneumoniae*
		• *Mycoplasma pneumoniae*

• ***Bordetella pertussis*** • ***Chlamydia pneumoniae*** • ***Mycoplasma pneumoniae***	• *Chlamydia pneumoniae* • *Mycoplasma pneumoniae*	

Clinical Utility and Validity of Panel Testing

The BioFire RP2.1 panel must be used with either the BioFire FilmArray 2.0 or BioFire FilmArray Torch Systems, and it does not provide a quantitative value for any organism within the sample. This panel "has not been established for specimens collected from individuals without signs or symptoms of respiratory infection" (BioFire, 2020). This panel has not been validated for the monitoring of treatment for any condition. If a test result shows four or more organisms detected, then the sample should be retested. A negative result does not necessarily exclude an infection. "Negative test results may occur from the presence of sequence variants (or mutation) in the region targeted by the assay, the presence of inhibitors, technical error, sample mix-up, an infection caused by an organism not detected by the panel, or lower respiratory tract infection that is not detected by a nasopharyngeal swab specimen" (BioFire, 2020).

The BioFire RP2.1 panel cannot necessarily distinguish between existing viral strains and new variants. One example is the inability to distinguish between Influenza A H3N2v and seasonal Influenza A H3N2. This panel also cannot reliably differentiate between human rhinovirus and enterovirus due to genetic similarity. If detected, the "result should be followed-up using an alternate method (e.g. cell culture or sequence analysis) if differentiation between the viruses is required" (BioFire, 2020). The performance characteristics of several microorganisms detected by this panel, including HCoV 229E, were determined using retrospective clinical specimens due to the small number of positive specimens collected. The BioFire RP2.1 panel should not be used if *B. pertussis* is suspected because of its low sensitivity. "[A] *B. pertussis* molecular test that is FDA-cleared for use on patients suspected of having a respiratory tract infection attributable to *B. pertussis* only should be used instead" (BioFire, 2020). This is because the RP2.1 panel targets a single-copy promoter target (*ptxP*) whereas more sensitive tests target the multi-copy IS*481* insertion sequence. The BioFire RP2.1 panel also shows cross-reactivity with *B. bronchiseptica* and *B. parapertussis* at higher concentrations.

The primers used in the BioFire RP2.1 panel to detect COVID-19 may cross-react with coronaviruses from other species due to high sequence homology. BioFire reports predicted cross-reactivity with up to three bat coronaviruses (accession: MN996532, MG772933, and MG772934) and one pangolin coronavirus (accession: MT084071). However, "[i]t is unlikely that these viruses would be found in a human clinical nasopharyngeal swab; but if present, the cross-reactive product(s) produced by the BioFire RP2.1 will be detected as Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)" (BioFire, 2020).

The difference between the BioFire RP2 panel and the BioFire RP2.1 panel is the ability to detect SARS-CoV-2. Consequently, within the Instructions for Use (IFU) for the RP2.1 panel, BioFire reports on the data of the RP2 panel. The clinical performance of the RP2 panel was determined using both fresh and frozen samples. The clinical performance values for the four endemic HCoVs are listed in **Table 2** (BioFire, 2020). They note a cross-reactivity between HCoV-OC43 and HCoV-HKU1.

Analyte	PPA	PPA 95% CI	NPA	NPA 95%CI
HCoV-229E	11/12 (91.7%)	64.6 – 98.5	1595/1600 (99.7%)	99.3 – 99.9

HCoV-HKU1	43/43 (100%)	91.8 – 100	1557/1569 (99.2%)	98.7 – 99.6
HCoV-NL63	40/40 (100%)	91.2 – 100	1562/1572 (99.4%)	98.8 – 99.7
HCoV-OC43	33/41 (80.5%)	66.0 – 89.8	1566/1571 (99.7%)	99.3 – 99.9
Notes: Abbreviations used—PPA (Positive Percent Agreement); NPA (Negative Percent Agreement).				

Concerning the detection of SARS-CoV-2, the BioFire RP2.1 panel reports a limit of detection (LoD), using the USA-WA1/2020 isolate, of 500 copies/mL when using a heat-inactivated virus. They report a 100% detection rate (20/20). This equates to 6.9 X 10^{-2} $TCID_{50}$/mL. They also tested the LoD using an infectious virus isolate obtained from the World Reference Center for Emerging Viruses and Arboviruses, contributed by the CDC. With this infectious sample, the LoD was determined to be 160 copies/mL (or 1.1 X 10^{-2} $TCID_{50}$/mL). Again, they report a 100% detection rate (20/20) (BioFire, 2020).

Similar to the BioFire panel test, the QIAstat-Dx Respiratory SARS-CoV-2 panel test by Qiagen is for use on a proprietary system, the QIAstat Dx Analyzer System. It is also a qualitative test approved for testing in "patients suspected of COVID-19 by their healthcare provider." It is also "not intended to be used as the sole basis for diagnosis, treatment, or other patient management decisions" (Qiagen GmbH, 2021). It is important to note that the test performance in either immunocompromised individuals or asymptomatic individuals has not been established as of publication date. A positive test result cannot rule out a co-infection; an erroneous negative test result can be due to erroneous sample handling as well as variations in the target sequences, organism levels below the limits of detection, and/or use of an interfering reagent (such as certain medications or therapies). Since the QIAstat-Dx test targets the *E* gene of SARS-CoV-2, which is homologous to sequences in multiple bat SARS viruses, it is possible to cross-react with these bat SARS viruses; however, the likelihood of infection of these viruses in humans is unlikely since none have been reported to date (Qiagen GmbH, 2021).

Also, like the BioFire RP2/RP2.1 panel tests, the QIAstat-Dx test may not distinguish between existing viral strains and emerging viral strains, such as influenza A. However, unlike the BioFire RP2/RP2.1 panel tests, the QIAstat-Dx test does detect the IS*481* multi-copy insertion sequence present in multiple *Bordetella* species. This does increase the sensitivity of the test, but it can increase the possibility of false-positive results if the specimen is contaminated with a non-pertussis *Bordetella* species (Qiagen GmbH, 2021).

In addressing the clinical performance of the QIAstat-Dx test for detecting SARS-CoV-2, Qiagen set up two positive trials (one at a higher concentration sample [n = 10] and one at a low positive contrived sample [n = 20), and they report a positive percent agreement (PPA) of 100% (30/30) (95% CI: 85.8 – 100%). Likewise, they did a negative control (n = 30) and report a negative percent agreement (NPA) of 100% (30/30) (95% CI: 85.8 – 100%). In reporting the limit of detection (LoD), they used 20 replicates with a detection rate of at least 95% (or 19/20) to generate a 'positive' signal. Using source material obtained from the clinical sample strain of the Hospital of Barcelona (Spain), Qiagen reports an LoD of 500 copies/mL.

The performance of the other targets within the panel were assessed in a multi-center study conducted at six geographically diverse study sites—Copenhagen, Denmark; Minneapolis, MN; Indianapolis, IN; Liverpool, NY; Columbus, OH; and Albuquerque, NM. The performance was determined using both frozen and fresh samples. The clinical performance values for the four endemic HCoVs are listed in **Table**

3 (Qiagen GmbH, 2021).

Table 3: Clinical Performance of QIAstat-Dx Panel for Endemic HCoVs				
Analyte	PPA	PPA 95% CI	NPA	NPA 95%CI
HCoV-229E	8/9 (88.9%)	56.5 – 98.0	1975/1975 (100%)	99.8 – 100.0
HCoV-HKU1	51/52 (98.1%)	89.9 – 99.7	1925/1932 (99.6%)	99.3 – 99.8
HCoV-NL63	40/47 (85.1%)	72.3 – 92.6	1936/1938 (99.9%)	99.6 – 100.0
HCoV-OC43	26/29 (89.7%)	73.6 – 96.4	1951/1955 (99.8%)	99.5 – 99.9
Notes: Abbreviations used—PPA (Positive Percent Agreement); NPA (Negative Percent Agreement).				

As with the other two tests, the ePlex RP2 Panel "should not be used as the sole basis for diagnosis, treatment, or other patient management decisions. Positive results are indicative of active infection with the identified respiratory pathogen but do not rule out infection or co-infection with non-panel organisms. The agent detected by the ePlex RP2 Panel may not be the definite cause of disease. Negative results for SARS-CoV-2 and other organisms on the ePlex RP2 Panel may be due to infection with pathogens that are not detected by this test, or lower respiratory tract infection that may not be detected by a nasopharyngeal swab specimen. Negative results do not preclude infection with SARSCoV-2 or other organisms on the ePlex RP2 Panel and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information" (GenMark Diagnostics, 2024). A limitation of ePlex RP2 Panel is its unpredictability in differentiating human rhinovirus and enterovirus due to genetic similarity. If differentiation is required, an ePlex RP2 Panel positive human rhinovirus/enterovirus result should be followed up using an alternative method, such as cell culture or sequence analysis. Cross-reactivity with SARS-CoV-1 is also observed at high titers.

To test the performance characteristics of ePlex RP2 Panel for SARS-CoV-2 detection, 170 nasopharyngeal previously frozen swab samples were collected (59 known SARS-CoV-2 positive and 111 presumed SARSCoV-2 negative samples). "Positive percent agreement (PPA) was calculated by dividing the number of true positive (TP) results by the sum of TP and false negative (FN) results, while negative percent agreement (NPA) was calculated by dividing the number of true negative (TN) results by the sum of TN and false positive (FP) results" (GenMark Diagnostics, 2024). The ePlex RP2 Panel detected SARS-CoV-2 in 59/59 positive specimens (100% positive percent agreement) and confirmed 111/111 negative specimens (100% negative percent agreement). To determine the limit of detection (LoD), the lowest concentration at which SARS-CoV-2 is detected at least 95% of the time, serial dilutions were prepared in a natural clinical matrix and at least 20 replicates per concentration were tested in the study. "The LoD concentration for detection of SARS-CoV-2 was determined to be 0.01 $TCID_{50}$/mL, which corresponds to 250 genomic copies per milliliter, as determined by digital droplet PCR" (GenMark Diagnostics, 2024).

Regarding the "Influenza SARS-CoV-2 (Flu SC2) Multiplex Assay" from the CDC, the FDA reported a limit of detection (LOD) of 1.01 x 10-2 (at ID50 [infective dose] / reaction). The panel was evaluated using 104 samples (33 positive for SARS-CoV-2, 30 positives for influenza A, and 30 positives for influenza B, 11 negative samples), and compared to an RT-PCR assay. There was a 100% concordance rate between the two tests. Additionally, cross-reactivity between the three analytes and 35 common respiratory pathogens (16 viruses, 18 bacterial species, one yeast) was evaluated, and no cross-reactivity was identified (FDA, 2024b).

The cobas SARS-CoV-2 & Influenza A/B panel is approved for emergency use authorization by the FDA; the panel uses qualitative detection of nucleic acids from SARS-CoV-2 in pooled samples. Six cultured viruses are tested for, two each of influenza A and influenza B strains as well as SARS-CoV-2. In an independent study, Poljak et al. (2020) performed a clinical evaluation of the cobas SARS-Cov-2 test (non-inclusive of influenza A/B panel). The cobas SARS-CoV-2 test was evaluated against an in house and well-characterized comparator using 217 samples. cobas and the comparator showed overall agreement of 98.1%. Another comparative evaluation on 502 samples showed agreement of 99.6%. The authors concluded that cobas "is a reliable assay for qualitative detection of SARS-CoV-2 in nasopharyngeal swab samples collected in the Universal Transport Medium System (UTM-RT)" (Poljak et al., 2020).

There are other panels that are not yet FDA approved such as the AMPLIQUICK® Respiratory Triplex assay that detects and differentiates between SARS-CoV-2, influenza A/B and respiratory syncytial viruses in respiratory specimens. Results from AMPLIQUICK® were compared to the Allplex™ Respiratory Panel 1 and 2019-nCoV assays. A total of 359 predetermined respiratory samples with diagnosed SARS-CoV-2, influenza A, influenza B and RSV were included in the study. The AMPLIQUICK® Respiratory Triplex "showed high concordance with the reference assays, with an overall agreement for SARS-CoV-2, influenza A, influenza B, and RSV at 97.6%, 98.8%, 98.3% and 100.0%, respectively." The authors conclude that the "AMPLIQUICK® Respiratory Triplex is a reliable assay for the qualitative detection and differentiation of SARS-CoV-2, influenza A, influenza B, and RSV in respiratory specimens, which may prove useful for streamlining diagnostics during the winter influenza-seasons" (Mboumba Bouassa et al., 2022).

Miscellaneous Testing

Other methodologies have been proposed to complement or even replace the standard tests described above. For example, a new "RT-LAMP" (reverse transcription loop-mediated isothermal amplification) application has started to see some use for the COVID-19 pandemic. This technique attempts to combine the speed of antigen testing and the accuracy of nucleic acid testing; RT-LAMP includes the traditional reverse transcriptase (RT), as well as a DNA polymerase with "strong strand displacement activity and tolerance for elevated temperatures and up to six DNA oligonucleotides of a certain architecture." These oligonucleotides act as primers for the RT, but additional oligonucleotides for the DNA polymerase are designed so that the DNA products loop back into their ends. This results in "self-priming templates" for the DNA polymerase, which allows the reaction [the nucleic acid amplification] to proceed as normal. Detection of the amplified DNA without specialized instrumentation is the key challenge; some tests use a pH indicator that changes the color of the solution the reaction is run in. Since the reaction does not require the use of a thermal cycler with real time fluorescence measurement, the results can be delivered in a faster time frame than traditional RT-PCRs (Dao Thi et al., 2020).

Nagura-Ikeda et al. (2020) evaluated the "clinical performance of six molecular diagnostic tests and a rapid antigen test for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)". Self-collected saliva was the medium used for analysis. A total of 103 patients with COVID-19 were included (15 asymptomatic, 88 symptomatic). The six molecular diagnostic tests included three RT-PCR tests, an RT-qPCR test, a "cobas SARS-CoV-2 high-throughput system" and an RT-LAMP assay. The molecular diagnostic tests detected viral RNA in 50.5%-81.6% of specimens and an antigen was detected in 11.7% of the specimens by the rapid antigen test. Viral RNA was also detected at a higher rate (65.6%-93.4%) in specimens collected within nine days of symptom onset compared to specimens collected after 10 days (22.2%-66.7%). Viral RNA was detected in asymptomatic patients at a rate of 40%-66.7%. The authors concluded "Self-collected saliva is an alternative specimen option for diagnosing COVID-19. LDT

RT-qPCR…and RT-LAMP showed sufficient sensitivity in clinical use to be selectively used according to clinical settings and facilities. The rapid antigen test alone is not recommended for initial COVID-19 diagnosis because of its low sensitivity" (Nagura-Ikeda et al., 2020).

Dao Thi et al. (2020) performed a validation of a "two-color RT-LAMP assay protocol for detecting SARS-CoV-2 viral RNA using a primer set specific for the N gene." The authors wrote that a positive sample would be detected by a color change from red to yellow and tested their RT-LAMP assay on "surplus RNA samples isolated from 768 pharyngeal swab specimens collected from individuals being tested for COVID-19." The results were compared to a traditional RT-qPCR assay. The specificity of the RT-LAMP assay was found to be 99.7%. Further, the RT-qPCR positive samples with a cycle threshold (CT) number of under 30 scored positive (agreeance) in the RT-LAMP assay at a 97.5% agreeance rate. Agreeance rate declined both at the 30-35 threshold and at the 35-40 threshold. The authors also developed a "swab-to-RT" LAMP protocol, which was measured at 86% sensitivity (for CT <30) and a 99.5% specificity. The authors concluded that "The RT-LAMP assay and LAMP-sequencing extend the range of available test methods and complement individual tests and pooled tests based on RT-qPCR with a faster, simpler, and potentially more cost-effective test method" (Dao Thi et al., 2020).

R. Wang et al. (2020) demonstrated a one-pot visual SARS-CoV-2 detection system named "opvCRISPR" by integrating reverse transcription loop-mediated isothermal amplification (RT-LAMP) and Cas12a cleavage in a single reaction system, which simplifies operations and avoids contamination. The opvCRISPR enables detection at every single molecular level in forty-five minutes. "The RT-LAMP reagents are incubated at the bottom of the tube, and CRISPR/Cas12a reaction reagents are added on the lid. SARS-CoV-2 RNA templates extracted from the respiratory swab are amplified by RT- LAMP, followed by mixing with the Cas12a reagents for cleavage. Once the Cas12a nuclease is activated by recognizing DNA target, it splits the quenched fluorescent single-stranded DNA (ssDNA) reporter (FAM-TTATT-BHQ1) indiscriminately, generating the fluorescence signal visible to the naked eye under blue light" (R. Wang et al., 2020). To investigate the diagnostic accuracy of opvCRISPR, 26 SARS-CoV-2 RT-PCR positive respiratory swab samples and 24 SARS-CoV-2 RT-PCR negative samples were tested. "All infected samples were determined to be SARS-CoV-2 positive while all uninfected samples tested to be negative by both opvCRISPR and RT- PCR. The opvCRISPR diagnostic results provide 100% agreement with the Centers for Disease Control and Prevention (CDC)-approved quantitative RT-PCR assay" (R. Wang et al., 2020). The author states that "the proposed method only requires minimal equipment, demonstrating great potential in enabling next-generation molecular diagnosis towards point-of- care diagnosis. However, the present method requires additional step to extract RNA. Further efforts need to be made to combine the RNA extraction module with the opvCRISPR to achieve from sampling to result nucleic acid detection" (R. Wang et al., 2020).

Another methodology with potential application for COVID-19 testing is next-generation sequencing (NGS). The NGS procedure typically includes the following steps: first the patient's DNA is prepared to serve as a template, then DNA fragments are isolated (on solid surfaces such as small beads) where sequence data is generated, then these results are compared against a reference genome. Any DNA sample may be used if the quality and quantity of that sample are sufficient, but the methods of library generation and data analysis often vary from panel to panel. NGS is often used to produce swift and high-volume sequencing (Hulick, 2024). The FDA issued an EUA to Illumina, Inc. for the Illumina COVIDSeq Test on June 10, 2020 but has since updated its indications on October 29, 2020 to be for the "qualitative detection of SARS-CoV-2 RNA from nasopharyngeal (NP) swabs, oropharyngeal (OP) swabs, anterior nasal swabs, mid-turbinate nasal swabs, nasopharyngeal wash/aspirates, nasal aspirates, and bronchoalveolar lavage (BAL) specimens from individuals suspected of COVID-19 by their healthcare provider" (FDA, 2021b). The FDA also issued an EUA to Helix OpCo LLC (dba Helix) for the Helix COVID-

19 NGS Test on August 6, 2020. The test detects the gene for the SARS-CoV-2 spike protein, as well as one internal control (the human gene *RPP30*). The limit of detection was found to be 125 genetic copy equivalents / mL, and both the positive and negative percent agreements were measured to be 100% over 30 samples (Helix, 2020).

Furthermore, whole genome sequencing (WGS) has been demonstrated to have application for COVID-19 testing as well. WGS is conducted through four steps of DNA shearing, by using "molecular scissors" to cut DNA; then DNA bar-coding, for which "scientists add small pieces of DNA tags, or bar codes to identify which piece of sheared DNA belongs to which [pathogen];" then the bar-coded DNA is put into the whole genome sequencer that identifies the bases; and finally, the data is analyzed to compare sequences and identify possible differences (CDC, 2024b). In several countries, like the Netherlands, China, Vietnam, and the United States, particularly rapid WGS has been beneficial in informing outbreak response, general public health decision making, and infection risk in various facilities (Chau et al., 2021; Oude Munnink et al., 2020; Taylor et al., 2020; F. Wang et al., 2020). In the Netherlands, WGS with the first cases in February 2020 was able to confirm separate introductions of the virus into the country, and attribute increases in case prevalence to co-circulating virus variants following the spring holidays. WGS informed the sequence diversity that existed in Italy, which was where most COVID-positive individuals were returning from. The researchers concluded that "WGS in combination with epidemiological data strengthened the evidence base for public health decision-making in the Netherlands as it enabled a more precise understanding of the transmission patterns in various initial phases of the outbreaks. As such, we were able to understand the genetic diversity of the multiple introduction events in phase 1, the extent of local and regional clusters in phase 2 and the transmission patterns within the HCW [healthcare worker] groups in phase 3 (among which the absence or occurrence of very limited nosocomial transmission)" (Oude Munnink et al., 2020). In Vietnam, a similar application was made regarding a previously known strain responsible for a virus outbreak in the northern region. By WGS, researchers were able to identify the first case of the B.1.1.7 variant from locally acquired infection. As the outbreak expanded, whole genome sequencing enabled enhanced surveillance in high risk groups, like those working in airports, who ended up being assigned another variant of A.23.1, as well as contact tracing and testing to detect more cases (Chau et al., 2021). In China, whole genome sequencing in this initial genomic study was able to provide insight towards the genotype-phenotype differences between COVID-19 positive patients. The researchers concluded, "Pedigree analysis suggested a potential monogenic effect of loss of function variants in GOLGA3 and DPP7 for critically ill and asymptomatic disease demonstration. Genome-wide association study suggests the most significant gene locus associated with severity were located in TMEM189–UBE2V1 that involved in the IL-1 signaling pathway…We identified that the HLA-A*11:01, B*51:01, and C*14:02 alleles significantly predispose the worst outcome of the patients" (F. Wang et al., 2020).

In the United States, a *Morbidity and Mortality Weekly Report (MMWR)* released in September 2020 utilized serial testing and virus whole genome sequencing at two skilled nursing facilities with COVID-19 outbreaks from April to June 2020 in Minnesota. From a total of 25 specimens from residents at the two different facilities, "strains from 17 residents and five HCP [health care personnel] were genetically similar, including one collected from a dietary worker with limited resident contact. Specimens from two HCP and one resident at facility A had distinctly different virus sequences from the first cluster and from each other. At facility B, 75 (66%) resident specimens and five (7%) HCP specimens were sequenced, all of which were genetically similar", which suggested "intrafacility transmission". However, the limited participation by HCPs in serial testing could have "have biased identification of infections and limited interpretation of genomic sequencing" and limited "the description of genetic diversity" (Taylor et al., 2020). Generally, whole genome sequencing still seems to have some limitations, in that "it still presents practical difficulties such as high cost, shortage of available reagents in the global market, need of a

specialized laboratorial infrastructure and well-trained staff" resulting in "SARS-CoV-2 surveillance blackouts across several countries" (Bezerra et al., 2021). As of May 4, 2022, there are no FDA approved tests specifically for WGS.

Other types of specimens or media have been proposed as viable for COVID-19 testing, such as saliva. Saliva's primary advantages include its flexibility, its safety, and overall ease of use in testing. Sri Santosh et al. (2020) also noted that To et al. (2019) found that saliva has a "high consistency rate of greater than 90% with nasopharyngeal specimens in the detection of respiratory viruses, including coronaviruses" (Sri Santosh et al., 2020; To et al., 2019). On August 15, 2020, the FDA issued an EUA to Yale School of Public Health for "SalivaDirect" which uses saliva samples for COVID-19 testing. Although this test still uses RT-PCR, the test still detects the nucleic acids in saliva, but does not require otherwise specialized or proprietary equipment for extraction of those nucleic acids. In the "Performance Evaluation" section of the official EUA, the FDA noted a positive agreement level between SalivaDirect and the ThermoFisher Scientific TaqPath COVID-19 combo kit to be 94.1% (32/34) and a negative agreement level to be 90.9% (30/33) (FDA, 2024a).

A third innovation in COVID-19 testing was published by the FDA on July 18, 2020. On this date, the FDA stated that they reissued an EUA to Quest Diagnostics to authorize Quest SARS-CoV-2 rRT-PCR test for use with "pooled" samples. This testing practice refers to testing multiple samples simultaneously, thereby allowing more efficient testing. The Quest SARS-CoV-2 rRT-PCR test was authorized to test up to four samples at once. The FDA notes that this strategy is most efficient in areas with low prevalence of COVID (i.e., most tests are expected to be negative). In the EUA, the FDA writes that if the "positivity rate" for any given individual to be tested is over 25%, the pooling strategy should not be used due to inefficiency (FDA, 2020). Yelin et al. (2020) found that a single positive sample could be identified in pools of up to 32 samples (with a false negative rate of 10%) and noted that detection of a single positive sample in a pool of 64 samples may be possible with additional amplification cycles (Yelin et al., 2020). Additional EUAs have been issued specifically for tests using pooled samples, such as the UCSD RC SARS-CoV-2 Assay (University of California San Diego Health, RT-PCR, five samples), the Poplar SARS-CoV-2 TMA Pooling assay (Poplar Healthcare, TMA [transcription-mediated amplification], seven samples), and the "COVID-19 RT-PCR Test" (LabCorp, RT-PCR, five samples) (LabCorp, 2022a; Poplar, 2020; UCSD, 2020).

Hogan et al. (2020) performed an analysis of pooled sample analysis in a community setting. The authors analyzed samples in pools of nine or 10, and the RT-PCR assay targeted the envelope (E) gene. When a positive pool was identified, each sample was tested individually for both the E gene and the RNA-dependent RNA polymerase (RdRp) gene for confirmation. The authors investigated 292 pools encompassing 2740 nasopharyngeal samples and 148 bronchoalveolar lavage samples. Two positive samples were identified (0.07%), which both showed detection of both genes. The authors identified one pool with a "positive E signal" that was not reproducible with testing individual samples of that pool. The authors did acknowledge that this methodology may miss individuals in which a COVID-19 risk has not been identified, but concluded that "strategies such as pooled screening may facilitate detection of early community transmission of SARS-CoV-2 and enable timely implementation of appropriate infection control measures to reduce spread (Hogan et al., 2020).

Guidelines and Recommendations

World Health Organization (WHO)

The World Health Organization (WHO) published an interim guideline for the diagnostic testing of "2019 novel coronavirus [termed 2019-nCoV]" on September 11, 2020 (WHO, 2020a). First, they state that routine confirmation of COVID-19 cases is based on nucleic acid testing. Regarding serum testing, they remark that "if negative NAAT results are obtained from a patient in whom SARS-CoV-2 infection is strongly suspected, a paired serum specimen could be collected. One specimen taken in the acute phase and one in the convalescent phase 2-4 weeks later can be used to look for seroconversion or a rise in antibody titres." Finally, they recommend against viral culture or isolation as a routine diagnostic procedure and WHO does not recommend the use of saliva as the sole specimen type for routine clinical diagnostics (WHO, 2020a).

The WHO released a scientific brief with recommendations for the use of SARS-CoV-2 Ag-RDTs and updated their interim guidance on October 6, 2021. Within the guidelines, "SARS-CoV-2 Ag-RDTs (antigen detecting rapid diagnostic tests) that meet the minimum performance requirements of ≥80% sensitivity and ≥97% specificity compared to a NAAT reference assay can be used to diagnose SARS-CoV-2 in suspected COVID-19 cases" (WHO, 2021a). Ag-RDTs should be conducted within five to seven days after the onset of symptoms, as "patients who present more than 5-7 days after the onset of symptoms are more likely to have lower viral loads, and the likelihood of false negative results with Ag-RDTs is higher." WHO recommends that Ag-RDTs be used in settings when they are most reliable – in areas "when there is ongoing community transmission (≥5% test positivity rate). When there is no transmission or low transmission, the positive predictive value of Ag-RDTs will be low (many false positives), and in this setting NAAT is preferable as the first-line testing method or for confirmation of positive Ag-RDTs" (WHO, 2021a).

The WHO recommends using SARS-CoV-2 Ag-RDTs when:

- "Symptomatic individuals (suspected COVID-19 cases) in the first 5-7 days since onset of symptoms"
- For asymptomatic individuals, only "limited to contacts of confirmed or probable cases and to at-risk health workers until more evidence is available on the benefits and cost effectiveness of testing low-risk groups with no known exposure to SARS-CoV-2, particularly in settings where testing capacity is limited."
- "Suspected COVID-19 cases in outbreak investigations" (WHO, 2021a).

The WHO indicates the following as priority uses for the Ag-RDTs:

- "Community testing of symptomatic individuals meeting the case definition of suspected COVID-19."
- "To detect and respond to suspected outbreaks of COVID-19 including in remote settings, institutions and semi-closed communities (e.g., schools, care-homes, cruise ships, prisons, workplaces and dormitories), especially where NAAT is not immediately available."
- "To screen asymptomatic individuals at high risk of COVID-19, including health workers, contacts of cases and other at-risk individuals" (WHO, 2021a).

Overall, "Ag-RDT testing is recommended in settings likely to have the most impact on early detection of cases for care and contact tracing and where test results are most likely to be correct" (WHO, 2021a).

The WHO released a second scientific brief with recommendations concerning immunity passports (WHO, 2020b) on April 24, 2020. Within the guidelines, WHO states that as of the publication date, "no study has evaluated whether the presence of antibodies to SARS-CoV-2 confers immunity to subsequent

infection by this virus in humans." They go on to note, "Laboratory tests that detect antibodies to SARS-CoV-2 in people, including rapid immunodiagnostic tests, need further validation to determine their accuracy and reliability. Inaccurate immunodiagnostic tests may falsely categorize people in two ways. The first is that they may falsely label people who have been infected as negative, and the second is that people who have not been infected are falsely labelled as positive. Both errors have serious consequences and will affect control efforts. These tests also need to accurately distinguish between past infections from SARS-CoV-2 and those caused by the known set of six human coronaviruses. Four of these viruses cause the common cold and circulate widely. The remaining two are the viruses that cause Middle East Respiratory Syndrome and Severe Acute Respiratory Syndrome. People infected by any one of these viruses may produce antibodies that cross-react with antibodies produced in response to infection with SARS-CoV-2" (WHO, 2020b).

In 2021, WHO released an update to the scientific brief concerning immunity passports within a document titled 'COVID-19 natural immunity.' Within this brief, WHO discusses the various testing methods available. WHO notes that "there are many available serologic assays that measure the antibody response to SARS-CoV-2 infection, but at the present time, the correlates of protection are not well understood." The most measured immune response is the presence of antibodies in serum. Serologic assays to detect the antibody response are usually based on enzyme immunoassays, which detect the presence of virus-specific antibodies in the blood or by live or pseudo-virus neutralization assays, which detect functional NAb. While serologic testing has limited use in clinical management because it does not capture active infection, it can be very useful in determining the extent of infection or estimating attack rates in given populations. Interpreting the results of serologic testing, however, is complex: there are several antibody types and subtypes and multiple antigenic determinants/epitopes that can be used to target these antibodies, and the results may differ substantially depending on the combinations chosen. The results will also depend on the manufacturing specifics of the assay used". Other frequently used assays are enzyme-linked immunosorbent tests, chemiluminescent tests, and lateral flow rapid diagnostic tests. To conclude, "available tests and current knowledge do not tell us about the duration of immunity and protection against reinfection, but recent evidence suggests that natural infection may provide similar protection against symptomatic disease as vaccination, at least for the available follow up period" (WHO, 2021c).

The WHO released guidelines for the use of SARS-CoV-2 antigen-detection rapid diagnostic tests for COVID-19 self-testing. The key points are:

- "COVID-19 self-testing, using SARS-CoV-2 Ag-RDTs, should be offered in addition to professionally administered testing services (Strong recommendation, low to moderate certainty evidence). This recommendation is based on evidence that shows users can reliably and accurately self-test, and that COVID-19 self-testing is acceptable and feasible and may reduce existing inequalities in testing access.
- The role and use of COVID-19 self-testing–including why, where and how it should be used–will need to be adapted to national priorities, epidemiology, resource availability, and local context with community input. Clear and up-to-date messaging will be needed to ensure self-test users can understand when to test, the meaning of their test results and post-test responsibilities.
- Self-testing should always be voluntary and never mandatory or coercive. It is important that in certain settings, such as schools and workplaces, self-testing costs are not borne by students or workers.
- Access to affordable and quality-assured SARS-CoV-2 Ag-RDTs, including for self-testing, should particularly be prioritized for settings where there is limited access to NAAT. COVID-19 self-test kits should meet the existing World Health Organization (WHO) standards for Ag-RDTs (≥ 80%

sensitivity and ≥ 97% specificity among symptomatic individuals).

- COVID-19 self-testing can be considered for both diagnostic and screening purposes. Depending on the epidemiological situation, a positive self-test result in symptomatic individuals or those with recent exposure could be used for diagnosis, and to facilitate linkage to clinical care and therapeutics.
- For screening purposes, a negative self-test result could enable participation in an activity, such as group activities or indoor gatherings, and confirmatory testing for positive results can be considered.
- Each country is facing a different situation in the pandemic depending on several factors including the intensity of SARS-CoV-2 circulation, amount of population level immunity, capacities to respond and agility to adjust measures. Timely and accurate diagnostic testing for SARS-CoV-2, the virus that causes COVID-19, is an essential part of a comprehensive COVID-19 response strategy. As the pandemic continues and the virus evolves, policy adjustments related to SARS-CoV-2 testing approaches and services, including COVID-19 self-testing, will be needed" (WHO, 2022).

The WHO released a scientific brief on May 15, 2020, concerning multisystem inflammatory syndrome in children and adolescents with COVID-19. Within the guidelines, they recommend standardized data describing clinical presentations.

- The WHO gives a preliminary case definition for individuals ages 0 – 19 years with fever three or more days AND at least TWO of the following:
  - "Rash or bilateral non-purulent conjunctivitis or muco-cutaneous inflammation signs (oral, hands or feet).
  - Hypotension or shock.
  - Features of myocardial dysfunction, pericarditis, valvulitis, or coronary abnormalities (including [echocardiogram] findings or elevated Troponin/NT-proBNP).
  - Evidence of coagulopathy (by PT, PTT, elevated d-Dimers).
  - Acute gastrointestinal problems (diarrhea, vomiting, or abdominal pain).
- AND
  - Elevated markers of inflammation such as ESR, C-reactive protein, or procalcitonin.
- AND
  - No other obvious microbial cause of inflammation, including bacterial sepsis, staphylococcal or streptococcal shock syndromes.
- AND
  - Evidence of COVID-19 (RT-PCR, antigen test or serology positive), or likely contact with patients with COVID-19" (WHO, 2020c).

Centers for Disease Control and Prevention (CDC)

In the CDC guidelines, Testing for COVID-19, there are two main types of viral tests used to detect current infections of SARS-CoV-2. Nucleic acid amplification tests (NAATs), which includes PCR tests, are the most highly recommended as they are highly sensitive and highly specific tests that detect one or more viral ribonucleic acid (RNA) genes. Viral RNA may stay in a person's body for up to 90 days after they test positive. Therefore, NAATs should not be used to test someone who has tested positive in the last 90 days (CDC, 2024f, 2024h).

Antigen tests are rapid tests that can produce results in 15-30 minutes. They are immunoassays that detect the presence of specific viral proteins, called antigens. Antigen tests generally have high

specificity, similar to NAATs, but are less sensitive than most NAATs. Therefore, "positive results are accurate and reliable. However, in general, antigen tests are less likely to detect the virus than NAAT tests, especially when symptoms are not present. Therefore, a single negative antigen test cannot rule out infection." The CDC recommends two negative antigen tests for individuals with symptoms or three antigen tests for those without symptoms, performed 48 hours apart to confirm an individual does not have COVID-19. However, a single NAAT test can be used to confirm an antigen test result (CDC, 2024f, 2024h).

- If an individual has not had COVID-19 or has not had a positive test within the past 90 days: they may choose a NAAT, including PCR, or antigen test. If the antigen test result is negative, repeat testing following the recommendations above.
- If an individual has tested positive for COVID-19 within the past 30 days or less with symptoms: use an antigen test. Repeat negative tests following the recommendations above.
- If an individual has tested positive for COVID-19 within the past 30 days or less with no symptoms: testing is not recommended to detect a new infection.
- If an individual has tested positive for COVID-19 within the 31-90 days with or without symptoms: use an antigen test. Repeat negative tests following the recommendations above (CDC, 2024h).

After a positive test result, you may continue to test positive for some time. Some tests, especially NAAT tests, may continue to show a positive result for up to 90 days. Reinfections can occur within 90 days, which can make it hard to know if a positive test indicates a new infection. Consider consulting a healthcare provider if you have any questions or concerns about your circumstances (CDC, 2024h).

Antibody (or serology) tests are used to test for the presence of antibodies from previous infection or vaccination and can be used in the diagnosis of Multisystem Inflammatory Syndrome in Children (MIS-C) or Multisystem Inflammatory Syndrome in Adults (MIS-A). However, antibody testing does not diagnose current infection. Antibody testing is not currently recommended to assess a person's protection against SARS-CoV-2 infection or severe COVID-19 following COVID-19 vaccination or prior infection, or to assess the need for vaccination in an unvaccinated person (CDC, 2024f).

In the CDC guidelines, MIS Case Definitions and Reporting, they define cases for MIS-C and MIS-A associated with SARS-CoV-2 infection. MIS is a rare but serious condition associated with SARS-CoV-2, in which different body parts become inflamed such as heart, lungs kidneys, brain, skin, eyes, and gastrointestinal tract. Children and adults with MIS experience ongoing fever PLUS more than one of the following: stomach pain, bloodshot eyes, diarrhea, dizziness or lightheadedness (signs of low blood pressure), skin rash, vomiting (CDC, 2024e). MIS-C is defined as any illness in a person <21 years of age that meets:

- "The clinical AND the laboratory criteria (Confirmed); OR
- The clinical criteria AND epidemiologic linkage criteria (Probable); OR
- The vital records criteria (Suspect)"

Clinical Criteria: An illness characterized by all of the following, in the absence of a more likely alternative diagnosis*

- "Subjective or documented fever (temperature ≥38.0^0 C)
- Clinical severity requiring hospitalization or resulting in death
- Evidence of systemic inflammation indicated by C-reactive protein ≥3.0 mg/dL (30 mg/L)
- New onset manifestations in at least two of the following categories:

    1. Cardiac involvement indicated by: Left ventricular ejection fraction <55% OR coronary artery dilatation, aneurysm, or ectasia, OR troponin elevated above laboratory normal range, or indicated as elevated in a clinical note

2. Mucocutaneous involvement indicated by: Rash, OR inflammation of the oral mucosa (e.g., mucosal erythema or swelling, drying or fissuring of the lips, strawberry tongue), OR conjunctivitis or conjunctival injection (redness of the eyes), OR extremity findings (e.g., erythema [redness] or edema [swelling] of the hands or feet)
3. Shock**
4. Gastrointestinal involvement indicated by: Abdominal pain, OR Vomiting, OR Diarrhea
5. Hematologic involvement indicated by: Platelet count <150,000 cells/uL, OR absolute lymphocyte count (ALC)"

Laboratory Criteria:

- "Detection of SARS-CoV-2 RNA in a clinical specimen*** up to 60 days prior to or during hospitalization, or in a post-mortem specimen using a diagnostic molecular amplification test (e.g., polymerase chain reaction [PCR]), OR
- Detection of SARS-CoV-2 specific antigen in a clinical specimen*** up to 60 days prior to or during hospitalization, or in a post-mortem specimen, OR
- Detection of SARS-CoV-2 specific antibodies^ in serum, plasma, or whole blood associated with current illness resulting in or during hospitalization"

Epidemiological Linkage Criteria: "Close contact‡ with a confirmed or probable case of COVID-19 disease in the 60 days prior to hospitalization."

Vital Records Criteria: "A person whose death certificate lists MIS-C or multisystem inflammatory syndrome as an underlying cause of death or a significant condition contributing to death"

"*If documented by the clinical treatment team, a final diagnosis of Kawasaki Disease should be considered an alternative diagnosis. These cases should not be reported to national MIS-C surveillance.
**Clinician documentation of shock meets this criterion.
***Positive molecular or antigen results from self-administered testing using over-the-counter test kits meet laboratory criteria.
^Includes a positive serology test regardless of COVID-19 vaccination status. Detection of anti-nucleocapsid antibody is indicative of SARS-CoV-2 infection, while anti-spike protein antibody may be induced either by COVID-19 vaccination or by SARS-CoV-2 infection
‡Close contact is generally defined as being within 6 feet for at least 15 minutes (cumulative over a 24-hour period). However, it depends on the exposure level and setting; for example, in the setting of an aerosol generating procedure in healthcare settings without proper personal protective equipment (PPE), this may be defined as any duration" (CDC, 2024e).

The CDC defines MIS-A as an illness in a person ≥ 21 years of age with:

- "Hospitalization for ≥ 24 hours* AND
- Subjective of documented fever (≥38.0 C) for ≥24 hours prior to hospitalization or within the first THREE days of hospitalization AND
- An illness meeting the following clinical and laboratory criteria:"

Clinical Criteria: "No alternative diagnosis (e.g. bacterial sepsis, exacerbation of a chronic medical condition) AND at least THREE of the following clinical criteria occurring prior to hospitalization or within the first THREE days of hospitalization. At least ONE must be a primary clinical criterion.

- Primary clinical criteria: Severe cardiac illness** (Includes myocarditis, pericarditis, coronary artery dilatation/aneurysm, new-onset right or left ventricular dysfunction (LVEF<50%), 2nd/3rd degree A-V block, or ventricular tachycardia). Rash AND non-purulent conjunctivitis

- Secondary clinical criteria: New-onset neurologic signs and symptoms (Includes encephalopathy in a patient without prior cognitive impairment, seizures, meningeal signs, or peripheral neuropathy including Guillain-Barré syndrome). Shock or hypotension not attributable to medical therapy (e.g., sedation, renal replacement therapy). Abdominal pain, vomiting, or diarrhea. Thrombocytopenia (platelet count <150,000/ microliter. "

Laboratory Criteria: "Evidence of SARS-CoV-2 infection (positive SARS-CoV-2 nucleic acid amplification (NAAT), serology, or antigen test) AND evidence of systemic inflammation (elevated levels of at least 2 of the following: C-reactive protein (CRP), ferritin, interleukin-6 (IL-6), erythrocyte sedimentation rate (ESR), procalcitonin). "

"*Or hospitalized for any length of time with an illness resulting in death
**Cardiac arrest alone does not meet this criterion" (CDC, 2024e).

According to the CDC, long COVID, also known as post-COVID conditions (PCC) is "an infection-associated chronic condition that can occur after SARS-CoV-2 infection and is present for at least 3 months as a continuous, relapsing and remitting, or progressive disease state that affects one or more organ systems" (CDC, 2024d). Long COVID is associated with:

- "Development of new or recurrent symptoms and conditions after the symptoms of initial acute COVID-19 illness have resolved.
- Symptoms that can emerge, persist, resolve, and reemerge over varying lengths of time.
- A spectrum of physical, social, and psychological consequences.
- Functional limitations that can affect patient wellness and quality of life"

Clinicians may clinically evaluate and diagnose Long COVID based on patient history and findings from a physical examination, while others might require directed diagnostic testing. Currently, no laboratory test can be used to definitively diagnose Long COVID or to distinguish Long COVID from conditions with different etiologies. Objective laboratory or imaging findings should not be used as the only measure or assessment of a patient's well-being. For example, a positive SARS-CoV-2 viral test or serologic (antibody) test are not required to establish a diagnosis of Long COVID but can help assess for current or previous infection.

A wide range of symptoms and clinical findings can occur in people with varying degrees of illness from acute SARS-CoV-2 infection. These effects can overlap with multiorgan complications, or with effects of treatment or hospitalization and can persist after the acute COVID-19 illness has resolved. While more than 200 Long COVID symptoms have been identified, commonly reported symptoms include:

- "Bloating/constipation/diarrhea
- Difficulty concentrating
- Light headedness/fast heart rate
- Memory change
- Persistent fatigue
- Post-exertional malaise
- Problems with smell
- Problems with taste
- Recurring headaches
- Shortness of breath/cough
- Sleep disturbance" (CDC, 2024d).

Post-exertional malaise (PEM) is the worsening of symptoms following even minor physical or mental exertion, with symptoms typically worsening 12 to 48 hours after activity and lasting for days or even weeks.

National Institutes of Health (NIH)

The NIH updated their COVID-19 treatment guidelines in May of 2024. The NIH addresses the clinical spectrum of SARS-CoV-2 infection, which includes those with asymptomatic or presymptomatic infection, mild illness, moderate illness, severe illness, and critical illness. For asymptomatic and presymptomatic individuals, the NIH states that "the percentage of individuals who present with asymptomatic infection and progress to clinical disease is unclear. Some asymptomatic individuals have been reported to have objective radiographic findings consistent with COVID-19 pneumonia." Additionally, the guideline discusses infectious complications in patients with COVID-19, which can be categorized as "coinfections at presentation," such as "concomitant viral infections, including influenza and other respiratory viruses" and community-acquired bacterial pneumonia, and "reactivation of latent infections," such as chronic hepatitis B virus and latent tuberculosis reactivation, "nosocomial infections," such as hospital-acquired or ventilator-associated pneumonia and *Clostridioides difficile*-associated diarrhea, and "opportunistic fungal infections," like aspergillosis and mucormycosis among hospitalized COVID-19 patients (NIH, 2024a).

The NIH also released COVID-19 testing guidelines. The following recommendations were made from the COVID-19 Treatment Guidelines Panel:

- The Panel recommends "using either a nucleic acid amplification test (NAAT) or an antigen test with a sample collected from the upper respiratory tract (e.g., nasopharyngeal, nasal mid-turbinate, or anterior nasal) to diagnose acute infection of SARS-CoV-2 (AIII)."
- "A NAAT should not be repeated in an asymptomatic person (with the exception of health care workers) within 90 days of a previous SARS-CoV-2 infection, even if the person has had a significant exposure to SARS-CoV-2."
- "SARS-CoV-2 reinfection has been reported in people after an initial diagnosis of the infection; therefore, clinicians should consider using a NAAT for those who have recovered from a previous infection and who present with symptoms that are compatible with SARS-CoV-2 infection if there is no alternative diagnosis (BIII)."
- "The Panel **recommends against** diagnosing acute SARS-CoV-2 infection solely on the basis of serologic (i.e., antibody) test results (AIII)."
- "There is insufficient evidence for the Panel to recommend either for or against the use of SARS-CoV-2 serologic testing to assess for immunity or to guide clinical decisions about using COVID-19 vaccines or anti-SARS-CoV-2 monoclonal antibodies" (NIH, 2024b).

American Medical Association (AMA)

The AMA released public health guidelines and recommendations concerning serological testing for SARS-CoV-2 antibodies on May 14, 2020. They list the limitations of antibody testing to include the potential for false-positive results, potential cross-reactivity, and lack of knowledge concerning relationship between antibody testing and immune status. The AMA recommends the following:

- "Use of serology tests should currently be limited to population-level seroprevalence study, evaluation of recovered individuals for convalescent plasma donations, and in other situations where they are used as part of a well-defined testing plan and in concert with other clinical information by physicians well-versed in interpretation of serology test results."
- "Serology tests should not be offered to individuals as a method of determining immune status."
- "Serology tests should not currently be used as the basis for any "immunity certificates," to inform decisions to return to work, or to otherwise inform physical distancing decisions. Doing so may

put individuals, their household and their community at risk."
- "Serology tests should not be used as the sole basis of diagnosis of COVID-19 infection" (AMA, 2020).

"Messaging on serological testing to medically underserved communities should explicitly take into consideration cultural and social features which may bear on their ability to make long-term choices on physical distancing and other COVID-19 precautions" (AMA, 2020).

Infectious Diseases Society of America (IDSA)

The IDSA released guidelines on the molecular diagnostic testing for COVID-19 which includes the following recommendations (IDSA, 2023):

"Recommendation 1: The IDSA panel recommends a SARS-CoV-2 NAAT in symptomatic individuals suspected of having COVID-19 (strong recommendation, moderate certainty evidence).

- Remarks:
  - The panel considered symptomatic patients to have at least one of the most common symptoms compatible with COVID-19 (Fever or chills, cough, shortness of breath or difficulty breathing, fatigue, muscle or body aches, headache, sore throat, new loss of taste or smell, congestion or runny nose, nausea or vomiting, diarrhea).
  - A positive test result may inform decisions about therapy, isolation, and potentially contact tracing.

There were limited data available regarding the analytical performance of SARS-CoV-2 NAATs in immunocompromised or vaccinated individuals, in those who have had prior SARS-CoV-2 infection, in children, or in patients infected with recent SARS-CoV-2 variants (e.g., Omicron).

Recommendation 2: For symptomatic individuals suspected of having COVID-19, the IDSA panel suggests collecting and testing swab specimens from either the nasopharynx (NP), anterior nares (AN), oropharynx (OP), or midturbinate regions (MT); saliva, or mouth gargle (conditional recommendation, low certainty evidence).

- Remarks:
  - Compared to NP swabs, AN or OP swabs alone yield more false-negative results than combined AN/OP swabs, MT swabs, saliva, or mouth gargle. Swabs of AN or OP alone are acceptable if collection of NP, AN/OP, or MT swabs, saliva, or mouth gargle is not feasible.
  - Sample collection methods are not standardized (e.g., drool or spit with/without cough were all reported as saliva)
  - . The patient's ability to follow instructions and cooperate with requirements of specimen collection (e.g., spit into a container, nothing by mouth for some time before saliva collection) should be considered.

FDA approval of individual NAATs specifically indicates collection and specimen type(s). Failure to adhere to label requirements, unless otherwise approved through a lab developed test (LDT) validation or authorized by the FDA through a subsequent EUA for different collection or specimen type, can lead to inaccurate results and reimbursement denials.

Recommendation 3: The IDSA panel suggests that for symptomatic individuals suspected of having COVID-19, AN and MT swab specimens may be collected for SARS-CoV-2 RNA testing by either patients or healthcare providers (conditional recommendation, moderate certainty evidence).

- Remarks:

An important limitation of the data available to inform this recommendation is that the type of specimen differed by comparison group. That is, while self-collected samples were always AN and MT specimens, healthcare provider-collected samples were always NP specimens. This might explain the increased sensitivity of healthcare provider collected specimens.

Recommendation 4: The IDSA panel suggests using either rapid or standard laboratory-based NAATs in symptomatic individuals suspected of having COVID-19 (conditional recommendation, moderate certainty of evidence).

- Remarks:
  - Appropriate specimen collection and transport to the laboratory or testing site are critical to ensuring high-quality results; resources are available on the IDSA website. Definitions of rapid NAATs have varied; some, including the U.S. FDA, consider turnaround times less than or equal to 30 minutes to define rapid NAATs, whereas others use less than or equal to 60-minutes or even longer. This time is for testing only (inclusive of nucleic acid extraction) and does not include time between specimen collection and testing or time between testing and reporting. Rapid tests typically have few operator steps and may be amendable to testing near patients or even at the point-of-care performed by non-laboratory staff. Rapid molecular test methodologies include rapid reverse transcription polymerase chain reaction (RT-PCR) and rapid isothermal NAAT. Standard tests require instrumentation and/or processing that must typically be performed in a clinical laboratory by trained laboratory staff.

This recommendation applies only to tests evaluated in the included studies. One test, Abbott IDNow, was included in most of the studies evaluated in this recommendation and may have skewed results towards lower sensitivity. Variability of test performance with different specimen types may be important. The evaluated assays used diverse technologies (e.g., isothermal and non-isothermal test amplification) that may theoretically impact results. Limited data were available regarding the analytical performance of NAATs in immunocompromised or vaccinated individuals, in those who have had prior SARS-CoV-2 infection, or in those infected with contemporary SARS-CoV-2 variants.

Recommendation 5: The IDSA panel suggests performing a single NAAT and not repeating testing routinely in symptomatic or asymptomatic individuals suspected of having COVID-19 whose initial NAAT result is negative (conditional recommendation, very low certainty of evidence).

- Remarks:
  - The panel considered symptomatic patients to have at least one of the most common symptoms compatible with COVID-19
  - . While repeat testing when the initial test result is negative is not suggested routinely, there may be situations where repeat testing might be considered. An example of such a situation is the development of new or worsening symptoms compatible with COVID-19 in the absence of an alternative explanation. Also, timing of symptom onset might drive a need for repeat testing. A poorly collected specimen could yield a falsely negative result and might be another reason for repeat testing.

If performed, repeat testing should generally occur 24-48 hours after initial testing and once the initial NAAT result has returned as negative.

Recommendation 6: For individuals who have clinical or epidemiologic reasons that might make testing desirable, the IDSA panel suggests SARS-CoV-2 RNA testing in asymptomatic individuals who are either

known or suspected to have been exposed to COVID-19 (conditional recommendation, moderate certainty evidence).

- Remarks:

The panel recognizes the lack of evidence supporting therapy for asymptomatic persons and the absence of treatment approved through EUA for asymptomatic COVID-19, but acknowledges that individual clinical scenarios may lead clinicians toward testing and consideration of treatment. Individuals who have clinical or epidemiologic reasons that might make testing desirable (e.g., high-risk individuals, such as those who have pulmonary conditions or are immunocompromised or those in close contact with immunocompromised individuals) may be considered for testing. Testing should be done at least 5 days after the exposure. If symptoms develop before 5 days, the exposed individual should get tested immediately[3]. Knowledge that an individual is infected with SARS-CoV-2 can be helpful to inform appropriate isolation. The decision to test asymptomatic persons should depend on the availability of testing resources. Known exposures are defined herein as close contact for at least 15 minutes over a 24-hour period with someone who has laboratory-confirmed COVID-19. Suspected exposures might be defined as working or residing in a congregate setting (e.g., long-term care or correctional facility, cruise ship, factory) experiencing a COVID-19 outbreak. The risk of contracting SARS-CoV-2 may vary under different exposure conditions, e.g., length of time exposed, indoor versus outdoor setting, whether masks were routinely worn. Household contacts may be especially high-risk. This recommendation assumes the exposed individual was not wearing appropriate PPE.

Recommendation 7: For individuals who have clinical or epidemiologic reasons that might make testing desirable, the IDSA panel suggests using either rapid or laboratory-based NAATs in asymptomatic individuals with known exposure to SARS-CoV-2 infection (conditional recommendation, moderate certainty of evidence).

- Remarks:
  - Appropriate specimen collection and transport to the laboratory or testing site are critical to ensure quality results; resources are available on the IDSA website. Definitions of rapid NAATs have varied; some, including the U.S. FDA, consider turnaround times less than or equal to 30 minutes to define rapid NAATs, whereas others use less than or equal to 60-minutes or even longer. This time is for testing only (inclusive of nucleic acid extraction) and does not include time between specimen collection and testing or time between testing and reporting. Rapid tests typically have few operator steps and may be amendable to testing near patients or even at the point-of-care performed by non-laboratory staff. Rapid test methodologies include rapid RT-PCR and rapid isothermal NAAT. Standard tests require instrumentation and/or processing that must typically be performed in a clinical laboratory by trained laboratory staff.

This recommendation applies only to tests evaluated in the included studies. Variability of test performance with different specimen types may be important. The evaluated assays used diverse technologies (e.g., isothermal and non-isothermal test amplification) that may theoretically impact results. Limited data were available regarding the analytical performance of NAATs in immunocompromised or vaccinated individuals, in those who have had prior SARS-CoV-2 infection, or in those infected with different SARS-CoV-2 variants.

Recommendation 8: The IDSA panel suggests against routine SARS-CoV-2 NAAT in asymptomatic individuals without a known exposure to COVID-19 who are being hospitalized (conditional recommendation, very low certainty evidence).

- Remarks:
  - Important considerations for this recommendation are that the IDSA panel was unable to identify studies published during the period of literature review that showed reduced SARS-CoV-2 transmission to healthcare providers or to other patients resulting from prehospitalization testing. The evidence was indirect and assessed only diagnostic test accuracy in studies of symptomatic patients alone or together with asymptomatic patients. The burden of testing all patients planned to be admitted was considered, in the face of limited evidence. Finally, there are other effective infection prevention interventions, including use of PPE and vaccination that should be considered.

The panel acknowledges that there could be a benefit of pre-admission NAAT in some situations, such as admission to a multibed room; to a unit with a congregate treatment area, such as a behavioral health unit; or to a positive pressure room or unit.

Recommendation 9: The IDSA panel suggests against routine SARS-CoV-2 NAAT of asymptomatic individuals without a known exposure to COVID-19 who are undergoing a medical or surgical procedure (conditional recommendation, very low certainty evidence).

- Remarks:
  - NAAT is used to determine presence of SARS-CoV-2 RNA, which may not represent infectious virus.
  - Detection of SARS-CoV-2 RNA in respiratory specimens without evidence of infectious virus has been reported widely.
  - The IDSA panel concluded that data were insufficient to establish SARS-CoV-2 infectiousness of a patient based on non-standardized instrument signal values, such as cycle threshold (Ct) values.
  - Decisions on the timing of a procedure in a patient with prior SARS-CoV-2 infection must balance the risk to the patient against the risks of delaying or avoiding the planned procedure, and should consider patient-related factors (e.g., vaccination status, symptomatic status, age), procedure-related factors (e.g., level of urgency, whether procedure generates aerosols), and procedural area infection control practices.
  - Given limited evidence for poor outcomes in asymptomatic persons who undergo major surgery soon after testing positive for SARS-CoV-2 infection, testing may be considered during periods of high community transmission.
  - Testing may also be considered before solid organ transplantation, hematopoietic stem cell transplantation or CAR-T cell therapy.

This recommendation applies to settings where protective measures, such as PPE, are available and are used with adherence. Other factors to consider include the vaccination status of healthcare providers and patients, and whether patients will be roomed with other patients before or after the procedure. This recommendation is based on general exposure in the community as compared to a specific known exposure.

Recommendation 10: The IDSA panel suggests against routinely repeating NAAT before medical or surgical procedures in patients with a recent history of COVID-19 (conditional recommendation, very low certainty evidence).

- Remarks:
  - NAAT is used to determine presence of SARS-CoV-2 RNA, which may not represent infectious virus.
  - Detection of SARS-CoV-2 RNA in respiratory specimens without evidence of infectious

virus has been reported widely.
    - Conversely, the IDSA panel was unable to find definitive evidence demonstrating that a negative NAAT result following a positive result is proof that a patient is no longer infectious.
    - The IDSA panel concluded that data were insufficient to establish SARS-CoV-2 infectiousness of a patient based on Ct value results.

Decisions on the timing of a procedure in a patient with prior SARS-CoV-2 infection must balance the risk to the patient against the risks of delaying or avoiding the planned procedure, and should consider patient-related factors (e.g., vaccination status, symptomatic status, age), procedure-related factors (e.g., level of urgency, whether procedure generates aerosols), and procedural area infection control practices.

Recommendation 11: The IDSA panel suggests against routinely repeating NAAT in patients with COVID-19 to guide release from isolation (conditional recommendation, very low certainty evidence).

- Remarks:
    - NAAT is used to determine presence of SARS-CoV-2 RNA, which may not represent infectious virus.
    - Detection of SARS-CoV-2 RNA in respiratory specimens for prolonged periods without evidence of infectious virus has been reported widely. Predicating release from isolation on a negative SARS-CoV-2 NAAT may extend the duration of isolation unnecessarily.
    - Conversely, the IDSA panel was unable to find definitive evidence demonstrating that a negative NAAT result following a positive result is proof that a patient is no longer infectious.

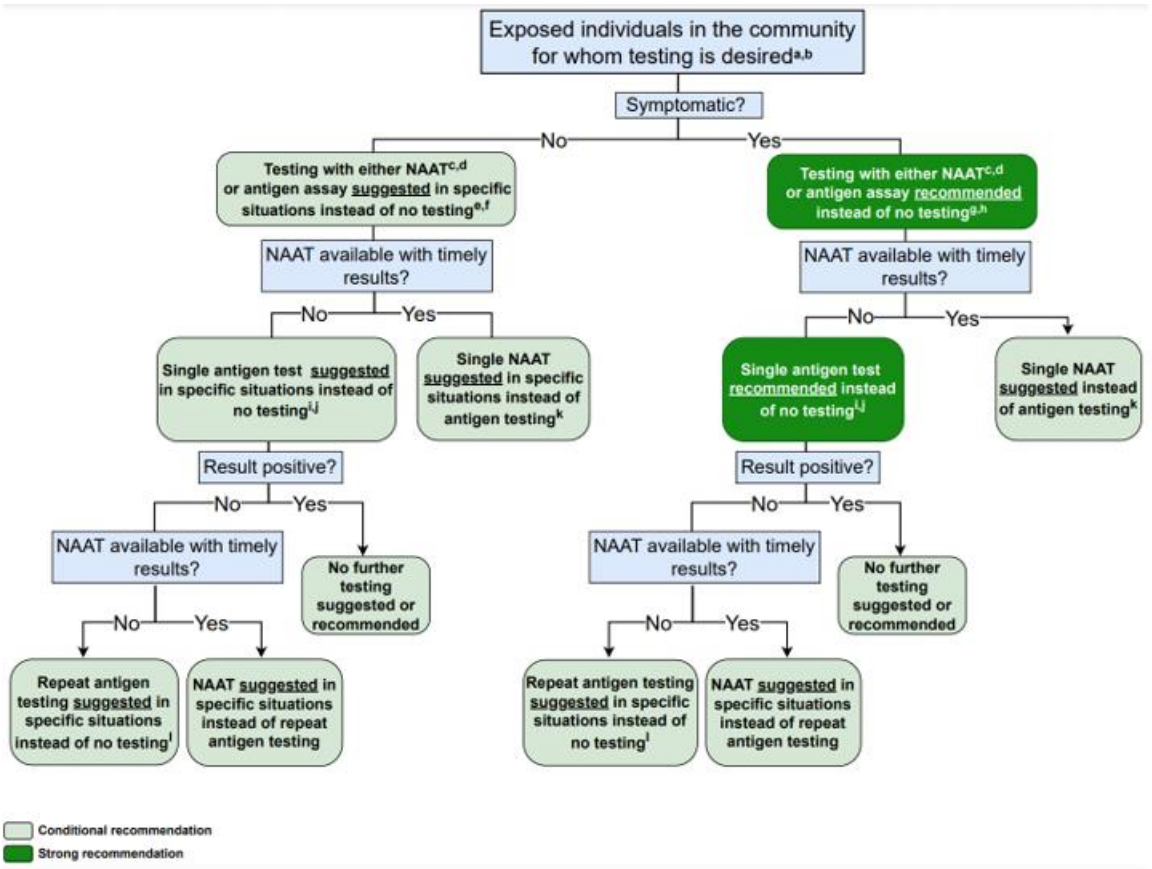
The IDSA panel concluded that data were insufficient to establish SARS-CoV-2 infectiousness of a patient based on Ct value results.

Recommendation 12: The IDSA panel suggests neither for nor against home-testing for SARS-CoV-2. (evidence gap).

- Remarks:
    - The panel defined time-sensitive surgery as medically necessary surgeries that need to be done within three months.
    - Testing should ideally be performed as close to the planned surgery as possible (e.g., within 48-72 hours).
    - To limit potential poor outcomes, deferring non-emergent surgeries should be considered for patients testing positive for SARS-CoV-2.
    - Decisions about PPE use for the aerosol generating portions of these procedures may be dependent on test results when there is limited availability of PPE. However, there is a risk for false negative test results, so caution should be exercised by those who will be in close contact with/exposed to the upper respiratory tract (e.g., anesthesia personnel, ENT procedures).

The decision to test asymptomatic patients will be dependent on the availability of testing resources. This recommendation does not address the need for repeat testing if patients are required to undergo multiple surgeries over time" (IDSA, 2023).

In total, the IDSA panel made 12 recommendations for SARS-CoV-2 nucleic acid testing based on new systematic reviews of the diagnostic literature. An updated algorithm based on these recommendations is provided to aid in decision-making seen below (IDSA, 2023).



The IDSA also published a guideline regarding serology testing with the following recommendations (IDSA, 2024):

- "The IDSA panel recommends against using serologic testing to diagnose SARS-CoV-2 infection during the first two weeks following symptom onset (strong recommendation, low certainty of evidence).
- The IDSA panel recommends against using IgG antibodies to provide evidence of COVID-19 in symptomatic patients with a high clinical suspicion and repeatedly negative NAAT (strong recommendation, very low certainty of evidence).
- To assist with the diagnosis of multisystem inflammatory syndrome in children (MIS-C), the IDSA panel recommends using both IgG antibody testing and NAAT to provide evidence of current or recent past COVID-19 (strong recommendation, very low certainty of evidence).
- When evidence of previous SARS-CoV-2 infection is desired, the IDSA panel suggests testing for SARS-CoV-2 IgG, IgG/IgM, or total antibodies three to five weeks after symptom onset and suggests against testing for SARS-CoV-2 IgM (conditional recommendation, low certainty of evidence).
- When evidence of prior SARS-CoV-2 infection is desired, the IDSA panel suggests using serologic assays that target nucleocapsid protein rather than spike protein (conditional recommendation, low

certainty of evidence).
- In individuals with previous SARS-CoV-2 infection or vaccination, the IDSA panel suggests against routine serologic testing given no demonstrated benefit to improving patient outcomes (conditional recommendation, very low certainty of evidence)" (IDSA, 2024).

Infectious Diseases Society of America (IDSA)/American Society for Microbiology (ASM)

In 2022, IDSA and ASM released a consensus review document on the clinical and infection prevention applications for SARS-CoV-2 genotyping. In it, they cover clinical use cases for genotyping, methods of genotyping, assay validation and regulatory requirements, clinical reporting for laboratories, and emerging issues in clinical SARS-CoV-2 sequencing. Overall, they report that "while clinical uses of SARS-CoV2 genotyping are currently limited, rapid technological change along with a growing ability to interpret variants in real time foretell a growing role for SARS-CoV-2 genotyping in clinical care as continuing data emerge on vaccine and therapeutic efficacy" (Greninger et al., 2022).

Society for Healthcare Epidemiology of America- (SHEA)/American Society of Anesthesiologists (ASA)/Anesthesia Patient Safety Foundation (APSF)

In late 2022, SHEA published recommendations on screening for SARS-CoV-2 in an asymptomatic population. Here, they note that testing of asymptomatic patients was an attempt to reduce the risk of nosocomial transmission but has been an extensive and resource intensive process with unclear benefit when added to other layers of infection prevention mitigation controls. They also note that "the logistic challenges and costs related to screening program implementation, data noting the lack of substantial aerosol generation with elective controlled intubation, extubation, and other procedures, and the adverse patient and facility consequences of asymptomatic screening call into question the utility of this infection prevention intervention." Based on their findings, SHEA "recommends against routine universal use of asymptomatic screening for SARS-CoV-2 in healthcare facilities. Specifically, preprocedure asymptomatic screening is unlikely to provide incremental benefit in preventing SARS-CoV-2 transmission in the procedural and perioperative environment when other infection prevention strategies are in place, and it should not be considered a requirement for all patients. Admission screening may be beneficial during times of increased virus transmission in some settings where other layers of controls are limited (eg, behavioral health, congregate care, or shared patient rooms), but widespread routine use of admission asymptomatic screening is not recommended over strengthening other infection prevention controls" (Talbot et al., 2023).

This statement is supported by the ASA and the APSF. They specifically note that the "SHEA recommendations provide a rationale for considering a move away from universal screening. Such a change considers the potential adverse consequences of testing for SARS-CoV-2 in asymptomatic patients. Moreover, we recommend that each facility develop a risk/benefit analysis that includes local/facility infection prevention assessment (e.g., patient population, facility physical layout, and community incidence and transmission of COVID-19 as defined in the SHEA Board Commentary), and a robust system of controls and interventions to prevent virus transmission ("Swiss Cheese" model). The recommendations by SHEA should be considered along with these updated recommendations to operationalize a robust and safe perioperative screening and targeted testing program for the benefit of our patients, our healthcare workers, other hospital patients and the public" (ASA & APSF, 2022).

American Association for Clinical Chemistry (AACC)

The AACC released a set of recommendations for "implementing and interpreting SARS-CoV-2 EUA and LDT serologic testing in clinical laboratories." Serologic testing is currently only used for serum, plasma, and "less frequently, whole-blood or dried blood spots," but not for other sample types, like saliva and cerebrospinal fluid. Serologic testing is "not recommended as the primary approach for diagnosis of SARS-CoV-2 infection." For the recommended use of serologic testing, the AACC stated the following:

- "Serologic testing may be offered as an approach to support diagnosis of COVID -19 illness in symptomatic patients and late phase negative molecular testing or for patients presenting with late complications such as multisystem inflammatory syndrome in children (MIS -C).
- Serologic testing can help identify people who may have been infected with or have recovered from the SARS -CoV -2 infection.
- Serologic testing can be used to screen potential convalescent plasma donors and in the manufacture of convalescent plasma.
- Serologic testing can be used for epidemiology and seroprevalence studies.
- Serologic testing can be used for vaccine response and efficacy studies."

Regarding serologic testing limitations, the AACC stated the following:

- "False positive results may occur.
- Negative results do not preclude acute SARS-CoV-2 infection or viral shedding.
- Serologic tests may not differentiate between natural infection and vaccine response.
- Serologic results should not be used for
    - Determining individual protective immunity
    - Return to work decisions
    - Cohorting individuals in congregate settings
    - Assessment of convalescent plasma recipients
    - Use of Personal Protective Equipment
    - Placement of high-risk job functions" (Zhang et al., 2021).

European Centre for Disease Prevention and Control (ECDC)

The ECDC in their guidance for laboratory support in the EU/EEA recommends using WHO-recommended testing strategies for the diagnosis and confirmation of COVID-19 (ECDC, 2023).

In the ECDC's guideline titled "COVID-19 testing strategies and objectives", the ECDC recommends performing laboratory testing in accordance with the WHO case definition. The following populations should be tested (ECDC, 2022b):

- "Ideally, all people with COVID-19 symptoms should be tested as soon as possible after symptom onset. This requires easy access to testing for all, including non-residents. Test result turnaround time should be minimized, people testing positive should isolate and timely contact tracing should be carried out, ensuring that all close contacts are tested, irrespective of symptoms.
- All patients with acute respiratory symptoms in hospitals and in other healthcare settings, and all specimens from sentinel primary care surveillance should be tested for both SARS-CoV-2 and influenza during the influenza season to monitor incidence and trends over time.
- Healthcare and social care settings require intensive testing when there is documented community transmission. Periodic and comprehensive testing of all staff and residents/patients is recommended to prevent nosocomial transmission. Furthermore, all patients/residents should be

tested upon or just prior to admission.

- Clusters or outbreaks may occur in certain settings, such as workplaces, educational facilities, prisons, and migrant detention centres. Testing policies and systems should be in place for rapid detection and control to protect the relevant populations in these settings and to protect the community from amplified transmission.
- Countries experiencing high SARS-CoV-2 transmission in a local community should consider testing the whole population of the affected area. This would enable identification of infectious COVID-19 cases and allow for their prompt isolation to interrupt chains of transmission. Depending on the epidemiological situation, size and population density of the affected area, such an approach could be less disruptive for society than having to introduce and ensure compliance with more stringent public health measures.
- To prevent re-introduction, countries or subnational areas that achieved sustained control of the circulation of SARS-CoV-2 should, in addition to quarantine measures, consider targeted testing and follow-up of individuals coming from other areas within the same country, or from other countries that have not yet achieved sustained control of the virus" (ECDC, 2022b).

The ECDC notes that "Genomic surveillance of SARS-CoV-2 is essential to detect, monitor and assess virus variants that can result in increased transmissibility, disease severity, or have other adverse effects on public health and social control measures. Obtaining timely and accurate information on the emergence and circulation of variants of concern (VOCs) and variants of interest (VOIs) requires robust surveillance systems, including integrated genome sequencing with a well-defined sampling and sequencing strategy to ensure representativeness and reliability of findings" (ECDC, 2021, 2022b).

The EDCD released guidelines on the use of antibody tests for SARS-CoV-2 in 2022. The key messages are:

- "At present, antibody tests are mostly used in research studies (mainly sero-epidemiological) at population level rather than for individual diagnosis of COVID-19 cases.
- A positive antibody test result can indicate a previous infection or vaccination but cannot be used to determine whether an individual is currently infectious or protected against infection.
- In the absence of a positive diagnostic test result, antibody tests cannot determine the time of infection.
- The antibody titres that correlate with protection from infection are currently unknown.
- There are a variety of antibody tests available and it is extremely difficult to compare their results due to the diversity and lack of standardisation.
- Antibody tests that target the spike protein are unable to distinguish between those who have been previously infected and those who have received at least one dose of a SARS-CoV-2 vaccine.
- There is a risk that the antibodies detected by the commercial tests currently in use will not prevent infection with newly emerging SARS-CoV-2 variants" (ECDC, 2022a).

American Academy of Pediatrics (AAP)

The AAP lists the most common scenarios for testing as symptomatic patients; patients who are asymptomatic but had exposure to a person with confirmed or probable COVID-19 infection; and patients who required screening as part of local public health, school, or workplace requirement. The AAP notes that a person's vaccination status may be a factor in decision-making concerning the need for screening (AAP, 2022).

Additionally, the AAP says that for patients who have symptoms, both NAATs (such as PCR testing) and antigen tests can be used. A positive result indicates a SARS-CoV-2 infection on either PCR or antigen diagnostics. That said, for a patient with a negative antigen result, a provider may repeat the antigen test at 48 hours per FDA guidance (AAP, 2022).

For purposes of testing symptomatic children who have recently had confirmed infections within three months, the AAP says providers should consider the possibility of a false-positive result. Especially using PCR tests and other NAAT tests, as these may remain positive from deposited viral genetic material for several months after an active infection. The AAP notes, "In a child with known exposure and compatible symptoms, there may be situations in which it is reasonable to retest within the 90-day window. If testing is performed within that window, antigen testing is generally preferable to NAATs because of the potential for positive NAAT results attributable to prior infection" (AAP, 2022).

Further, the AAP previously stated in 2020-2021 guidance that antibody (serologic) tests "can provide evidence of previous infection with SARS-CoV-2 but are not useful for the diagnosis of acute infection. A positive antibody test result does not prove that a patient has protection against SARS-CoV-2, although the FDA and vaccine companies use serologic testing as a marker for immunogenicity and protection from SARS-CoV-2 infection. Thus, these tests should not be used to make decisions on grouping people in classrooms or other facilities at this time, and individuals with positive antibody tests should continue to adhere to guidelines about masking, social distancing, and other preventive measures" (AAP, 2022).

The AAP has also included some comments and discussion on Multisystem Inflammatory Syndrome in Children (MIS-C). MIS-C has been observed to have some association with COVID-19, and patients with this syndrome have been observed to test positive "far more often" for past SARS-CoV-2 infection (i.e., antibody testing) than acute infection (RT-PCR or antigen test). The Council of State and Territorial Epidemiologists (CSTE) and CDC defines an MIS-C case by the following criteria:

"An individual aged <21 years and in the absence of a more likely alternative diagnosis:

- Subjective or documented fever (T >38.0° C)
- Clinical severity requiring hospitalization or resulting in death
- C-reactive protein (CRP) >3.0 mg/dL
- New onset manifestations of >2 of the following categories:
    - Cardiac: coronary artery dilatation/aneurysm, left ventricular ejection fraction <55%, or troponin elevated above normal
    - Shock
    - Mucocutaneous: rash, oral mucosal inflammation, conjunctivitis/conjunctival injection or extremity findings (erythema, edema)
    - Gastrointestinal: abdominal pain, vomiting or diarrhea
    - Hematologic: platelet count <150,000/µL, absolute lymphocyte count <1000/µL
- Detection of SARS-CoV-2 nucleic acid/antigen up to 60 days prior to or during hospitalization or in a postmortem specimen, OR detection of antibody associated with current illness, OR close contact with a confirmed/probable COVID-19 case in the 60 days prior to hospitalization" (AAP, 2023).

The CDC delineates a testing algorithm for MIS-C in the outpatient or emergency department setting as follows:

- "Evaluate a child with persistent fever (≥3 days) who is moderately to severely ill with clinical signs of organ dysfunction (eg, gastrointestinal, respiratory, cardiac, mucocutaneous or hematologic). Initial evaluation should include measurement of vital signs, assessment of perfusion and oxygen saturation. Early consultation and coordination with the nearest pediatric infectious disease and rheumatology specialist and pediatric referral center for optimal testing and management should be considered. Laboratory screening for systemic inflammation may be considered and initial lab screenings may include complete blood cell count (CBC) with differential, urine analysis, ESR, and CRP, with the addition of ferritin, LDH, comprehensive metabolic panel, pro-BNP, troponin and fibrinogen depending on initial clinical suspicion and/or evidence of inflammation on initial lab screening. Note that none of these laboratory studies is specific for the diagnosis of MIS-C, so even if there is evidence of significant systemic inflammation, alternative diagnoses must still be considered (eg, pyelonephritis, appendicitis)" (AAP, 2023).

For the evaluation of severely ill appearing or hemodynamically fragile patients, they propose that:

> "Severely ill-appearing patients and those in compensated shock or shock should be evaluated and treated in the emergency department/critical care setting. Transfer to a referral center should be arranged. Laboratory tests, as described above, should be performed for initial evaluation regardless of duration of fever. Consultation with pediatric subspecialists (infectious diseases, cardiology, rheumatology) at a local or regional pediatric referral center should be initiated but should not delay transfer to a referral center" (AAP, 2023).

Testing for hospitalized children is delineated below.

"Any child sick enough to warrant admission for fever, abdominal pain, diarrhea and/or organ dysfunction in whom MIS-C is suspected should be cared for in a hospital with tertiary pediatric/cardiac intensive care units. Although decisions about additional testing will be made by the multidisciplinary team managing the patient, pediatricians can prepare families for an expanded laboratory and cardiac workup that may include:

- Chest radiograph, EKG and troponin. If any of these or physical examination is abnormal, then consult with pediatric cardiology and consider additional diagnostic testing for myocardial injury (echocardiogram and/or cardiac MRI).
- Expanded laboratory tests including pro-BNP, triglycerides, creatine kinase, amylase, blood and urine culture, D-dimer, prothrombin time/partial thromboplastin time (PT/PTT), INR, CRP, ferritin, LDH, comprehensive metabolic panel and fibrinogen, if not already conducted.
- In all cases, COVID-19 testing should be performed with RT-PCR assay and serologic testing. Later serology may be needed if all are negative initially. Serologic tests must be sent prior to administration of intravenous immunoglobulin (IVIG)" (AAP, 2023).

American College of Rheumatology (ACR)

The ACR published guidance regarding MIS-C associated with COVID-19. In it, they list SARS-CoV-2 IgG, IgM, and IgA as part of the diagnostic pathway for MIS-C (Henderson, Canna, Friedman, Gorelik, Lapidus, Bassiri, Behrens, Ferris, Kernan, Schulert, Seo, MB, et al., 2020).

In a December 5, 2020 update of the above guidelines, the ACR states that ESR, CRP, and testing for SARS-CoV-2 (by PCR or serology) should be considered a "tier 1" (first-line evaluation) for MIS-C

(Henderson, Canna, Friedman, Gorelik, Lapidus, Bassiri, Behrens, Ferris, Kernan, Schulert, Seo, Son, et al., 2020).

In a February 3, 2022 update of the above guideline, the ACR added new information concerning immunomodulatory treatment in MIS-C, hyperinflammation in COVID-19, as well as statements on thrombotic risk and anticoagulation in MIS-C (Henderson et al., 2022).

Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, please visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

The FDA issued an "Immediately in Effect Guidance on policy for diagnostics testing in laboratories certified to perform high complexity testing under CLIA prior to Emergency Use Authorization for Coronavirus Disease-2019 during the public health emergency" in February 2020 (FDA, 2024c). This policy was updated on May 11, 2020 to state that the "policy is intended to remain in effect only for the duration of the public health emergency related to COVID-19 declared by the Secretary of Health and Human Services (HHS) on January 31, 2020, effective January 27, 2020, including any renewals made by the HHS Secretary in accordance with section 319(a)(2) of the Public Health Service Act (PHS Act)" (FDA, 2023b). As of October 15, 2021, the FDA had issued 418 different EUAs for COVID-19 testing for either *in vitro* diagnostic products (which includes testing such as point-of-care tests, antibody testing, and antigen testing) or high complexity molecular-based laboratory developed tests (FDA, 2021a).

Moreover, within the HR 748, passed as the CARES Act (or Coronavirus Aid, Relief, and Economic Security Act) as public law 116-136 on March 27, 2020, there are sections concerning coverage and pricing of diagnostic testing for COVID-19 (US, 2020).

In March 2023, the FDA released a "transition plan for medical devices that fall within enforcement policies issued during the coronavirus disease 2019 (COVID-19) public health emergency" and a "transition plan for medical devices issued emergency use authorizations (EUAs) related to coronavirus disease 2019 (COVID-19)." These guidelines are meant to outline the FDA's recommendations during the transition from the COVID-19 pandemic to normal operations (FDA, 2023c, 2023d).

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
86318	Immunoassay for infectious agent antibody(ies), qualitative or semiquantitative, single step method (eg, reagent strip)
86328	Immunoassay for infectious agent antibody(ies), qualitative or semiquantitative, single step method (eg, reagent strip); severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Coronavirus disease [COVID-19])
86408	Neutralizing antibody, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Coronavirus disease [COVID19]); screen
86409	Neutralizing antibody, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Coronavirus disease [COVID19]); titer
86413	Severe acute respiratory syndrome coronavirus 2 (SARSCoV-2) (Coronavirus disease [COVID-19]) antibody, quantitative
86769	Antibody; severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Coronavirus disease [COVID-19])
87426	Infectious agent antigen detection by immunoassay technique, (eg, enzyme immunoassay [EIA], enzyme-linked immunosorbent assay [ELISA], fluorescence immunoassay [FIA], immunochemiluminometric assay [IMCA]) qualitative or semiquantitative; severe acute respiratory syndrome coronavirus (eg, SARS-CoV, SARS-CoV-2 [COVID-19])
87428	Infectious agent antigen detection by immunoassay technique, (eg, enzyme immunoassay [EIA], enzyme-linked immunosorbent assay [ELISA], fluorescence immunoassay [FIA], immunochemiluminometric assay [IMCA]) qualitative or semiquantitative; severe acute respiratory syndrome coronavirus (eg, SARS-CoV, SARS-CoV-2 [COVID-19]) and influenza virus types A and B
87635	Infectious agent detection by nucleic acid (DNA or RNA);severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Coronavirus disease [COVID-19]), amplified probe technique
87798	Infectious agent detection by nucleic acid (DNA or RNA), not otherwise specified; amplified probe technique, each organism
87811	Infectious agent antigen detection by immunoassay with direct optical (ie, visual) observation; severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Coronavirus disease [COVID-19])
87913	Infectious agent genotype analysis by nucleic acid (DNA or RNA); severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (coronavirus disease [COVID-19]), mutation identification in targeted region(s)
0224U	Antibody, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Coronavirus disease [COVID-19]), includes titer(s), when performed Proprietary test: COVID-19 Antibody Test Lab/Manufacturer: Mount Sinai Laboratory/Mt Sinai
0226U	Surrogate viral neutralization test (sVNT), severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Coronavirus disease [COVID-19]), ELISA, plasma, serum Proprietary test: Tru-Immune™ Lab/Manufacturer: Ethos Laboratories/GenScript® USA Inc
0408U	Infectious agent antigen detection by bulk acoustic wave biosensor immunoassay, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (coronavirus disease [COVID-19]) Proprietary test: Omnia™ SARS-CoV-2 Antigen Test Lab/Manufacturer: Qorvo Biotechnologies

CPT	Code Description
U0001	CDC Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel
U0002	Non-CDC laboratory test for 2019-nCoV (COVID-19), any method

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

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Revision History

Revision Date	Summary of Changes
12/04/2024	Reviewed and Updated: Updated background, guidelines, and evidence-based scientific references. Literature review necessitated the following changes in coverage criteria: Added NAAT as an acceptable test option for MIS-A and MIS-C, now reads: "4) To support a diagnosis of multisystem inflammatory syndrome in children (MIS-C) (see Note 2), multisystem inflammatory syndrome in adults (MIS-A) (see Note 3), or post-acute sequelae of SARS-CoV-2 infection (PASC), nucleic acid amplification testing and host antibody serology testing MEET COVERAGE CRITERIA." Updated CC5 to include a once every 48-hour frequency, now reads: "5) For symptomatic individuals, antigen-detecting diagnostic tests for SARS-CoV-2 (e.g., antigen rapid tests) once every 48 hours MEET COVERAGE CRITERIA." Removed CC7 and CC9 due to redundancy with G2149-Pathogen Panel Testing. Multiplex PCR testing for respiratory pathogens is more appropriately managed by the Pathogen Panel Testing policy and is not needed in this (G2174) policy. "7) For individuals with signs and symptoms of a respiratory tract infection (see Note 4), multiplex PCR-based panel testing of up to 5 respiratory pathogens MEETS COVERAGE CRITERIA. 9) Multiplex PCR-based panel testing of 6 or more respiratory pathogens DOES NOT MEET COVERAGE CRITERIA." Updated Note 1 with updated CDC signs and symptoms of COVID-19. Updated Note 2 and Note 3 with updated CDC clinical requirements for suspected MIS-C and MIS-A.

	Removed CPT code 87631, 87632, 87633, 0115U, 0202U, 0223U, 0225U; deleted code C9803 (effective date 01/01/2024)
09/06/2023	Off-cycle coding modification: Added CPT code 0408U (effective date 10/01/2023)

Diabetes Mellitus Testing

Policy Number: AHS – G2006 – Diabetes Mellitus Testing	Initial Presentation Date: 9/18/2015 Effective Date: 4/1/2025

Policy Description

Diabetes describes several heterogeneous diseases in which various genetic and environmental factors can result in the progressive loss of β-cell mass and/or function that manifests clinically as hyperglycemia (Skyler et al., 2017).

Fasting plasma glucose (FPG) and oral glucose tolerance testing (OGTT) can be used in the diagnosis of diabetes mellitus. FPG is obtained from blood after a typically overnight period of not eating, whereas the OGTT is performed to understand an individual's response to a concentrated solution of glucose after two hours, typically in the setting of pregnancy (MayoClinic, 2024). In an asymptomatic individual, FPG ≥ 126 mg/dL or two-hour plasma glucose values of ≥200 mg/dL during a 75 g OGTT establish a diagnosis of diabetes. In reference to A1c values, individuals with percentages 5.7 to <6.5% are at highest risk. Additionally, there is a continuum of increasing risk amongst individuals with A1c levels <6.5% (Inzucchi & Lupsa, 2023). These assays are identified to be affordable alternatives to the more costly yet more convenient HbA1c level, and are more often used in the diagnosis of type 2 diabetes mellitus (Hayward & Selvin, 2023).

Glycated hemoglobin (A1c) results from post-translational attachment of glucose to the hemoglobin in red blood cells at a rate dependent upon the prevailing blood glucose concentration. Therefore, these levels correlate well with glycemic control over the previous eight to twelve weeks (Selvin, 2022). The

measurement of hemoglobin A1c is recommended for diabetes management, including screening, diagnosis, and monitoring for diabetes and prediabetes.

Terms such as male and female are used when necessary to refer to sex assigned at birth.

Related Policies

Policy Number	Policy Title
AHS-G2035	Prenatal Screening (Nongenetic)

Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) For individuals with acute or persistent classic symptoms of diabetes mellitus, measurement of plasma glucose **MEETS COVERAGE CRITERIA**.

2) For individuals with a diagnosis of either type 1 or type 2 diabetes mellitus, measurement of hemoglobin A1c **MEETS COVERAGE CRITERIA** in **any** of the following situations:
   a) Upon initial diagnosis to establish a baseline value and to determine treatment goals.
   b) Twice a year (every 6 months) in individuals who are meeting treatment goals and who, based on daily glucose monitoring, appear to have stable glycemic control.
   c) Quarterly in individuals who are not meeting treatment goals for glycemic control.
   d) Quarterly in individuals whose pharmacologic therapy has changed.
   e) Quarterly for individuals who are pregnant.

3) For prediabetic individuals, annual screening for type 2 diabetes with a fasting plasma glucose test **or** measurement of hemoglobin A1c **MEETS COVERAGE CRITERIA**.

4) For asymptomatic individuals who are 35 years of age or older and who have no risk factors for diabetes, screening for prediabetes or type 2 diabetes once every three years with a fasting plasma glucose test **MEETS COVERAGE CRITERIA**.

5) For individuals 18 years of age or older, screening once every three years for prediabetes or type 2 diabetes with a fasting plasma glucose test or measurement of hemoglobin A1c **MEETS COVERAGE CRITERIA** for individuals with **any** of the following risk factors:
   a) For individuals who are overweight or obese.
   b) For first-degree relatives (see Note 1) of individuals with diabetes.
   c) For individuals with a history of cardiovascular disease.
   d) For individuals with hypertension.
   e) For individuals with hypercholesterolemia.

f) For individuals with metabolic syndrome.

g) For individuals who are obese and have acanthosis nigricans.

h) For individuals with polycystic ovary syndrome.

i) For individuals with metabolic dysfunction-associated steatotic liver disease (MASLD).

j) For individuals who were previously diagnosed with gestational diabetes mellitus (GDM).

6) For individuals who are positive for HIV, screening for diabetes and prediabetes with a fasting plasma glucose test **MEETS COVERAGE CRITERIA** in **any** of the following situations:

   a) For individuals starting antiretroviral therapy (ART).

   b) For individuals switching their ART.

   c) 3-6 months after starting or switching antiretroviral therapy.

   d) Annually when screening results were initially normal.

7) For individuals 10 years of age and older who have been diagnosed with cystic fibrosis (CF) but not with CF-related diabetes, annual screening for CF-related diabetes with an OGTT **MEETS COVERAGE CRITERIA**.

8) For overweight or obese individuals less than 18 years of age, diabetes screening once every three years with a fasting plasma glucose test, an OGTT, **or** measurement of hemoglobin A1c **MEETS COVERAGE CRITERIA** for individuals with **any** of the following risk factors:

   a) The individual has a maternal history of diabetes or gestational diabetes mellitus during the child's gestation.

   b) The individual has a family history of type 2 diabetes in first- or second-degree relatives (see Note 1).

   c) The individual has signs of insulin resistance or conditions associated with insulin resistance (acanthosis nigricans, hypertension, dyslipidemia, polycystic ovary syndrome, or small-for-gestational-age birth weight).

9) For pregnant individuals, a fasting plasma glucose test or an OGTT up to once per month during pregnancy **MEETS COVERAGE CRITERIA**.

10) For individuals diagnosed with GDM during pregnancy, an OGTT **MEETS COVERAGE CRITERIA** in **any** of the following situations:

   a) To screen for persistent diabetes or prediabetes 4-12 weeks postpartum.

   b) For individuals with a positive initial postpartum screening result, repeat screening to confirm a diagnosis of persistent diabetes or prediabetes.

11) For all other situations not addressed above, fasting plasma glucose testing at a wellness visit with no abnormal findings **DOES NOT MEET COVERAGE CRITERIA**.

12) For all other situations not previously described (see Note 2), measurement of hemoglobin A1c **DOES NOT MEET COVERAGE CRITERIA**.

NOTES:

Note 1: First-degree relatives include parents, full siblings, and children of the individual. Second-degree relatives include grandparents, aunts, uncles, nieces, nephews, grandchildren, and half-siblings of the individual.

Note 2: Measurement of hemoglobin A1c **should not** be performed in **any** of the following situations:

1) To test for diabetes in individuals presenting with acute or persistent classic symptoms of diabetes mellitus.
2) In pregnant individuals without an established diagnosis of diabetes or prediabetes.
3) To screen for diabetes in individuals diagnosed with cystic fibrosis.
4) In conjunction with measurement of fructosamine.
5) In individuals with a condition associated with increased red blood cell turnover (e.g., individuals with sickle cell disease or who are HIV positive, individuals receiving hemodialysis or erythropoietin therapy or who have had recent blood loss or a transfusion).

Table of Terminology

Term	Definition
1,5AG	1,5-Anhydroglucitol
2-h PG	2-h plasma glucose
A1c	Glycated hemoglobin
AACE	American Association of Clinical Endocrinologists
AAFP	American Academy of Family Physicians
ACE	American College of Endocrinology
ACP	American College of Physicians
ADA	American Diabetes Association
aRR	Adjusted risk ratios
ARV	Antiretroviral
BMI	Body mass index
BP	Blood pressure
CAP	College of American Pathologists
CF	Cystic fibrosis
CFPD	Cystic fibrosis-related prediabetes
CFRD	Cystic fibrosis-related diabetes
CHF	Congestive heart failure
CKD	Chronic kidney disease
CMS	Centers For Medicare and Medicaid Services
COVID-19	Coronavirus 19
CV	Coefficient of variation
CVA	Cerebrovascular accident
CVD	Cardiovascular disease
DCCT	Diabetes Control and Complications Trial
FA	Fructosamine
FDA	Food and Drug Administration
FPG	Fasting plasma glucose
GA	Glycated albumin

GCT	Glucose challenge test
GDM	Gestational diabetes mellitus
HbA1c	Hemoglobin A1C/Glycated hemoglobin
HDL	High-density lipoprotein
HIV/AIDS	Human immunodeficiency virus, acquired immunodeficiency syndrome
HPLC	High-performance liquid chromatography
IFCC	International Federation of Clinical Chemistry
IFG	Impaired fasting glucose
IGT	Impaired glucose tolerance
IHD	Ischemic heart disease
ISPAD	International Society for Pediatric and Adolescent Diabetes
KDIGO	Kidney Disease: Improving Global Outcomes Diabetes Working Group
LDTs	Laboratory-developed tests
MACE	Major adverse cardiovascular events
MASLD	Metabolic dysfunction-associated steatotic liver disease
MODY	Maturity-onset diabetes of the young
NACB	National Academy of Clinical Biochemistry
NGSP	National Glycohemoglobin Standardization Program
NICE	National Institute for Health and Care Excellence
OGTT	Oral glucose tolerance test
OR	Odds ratio
POC	Point-of-care
ROC-AUC	Receiver operative characteristic, area under the curve
SES	Socioeconomic status
SMBG	Self-monitoring of blood glucose
T1D	Type 1 Diabetes
TIA	Transient ischemic attack
USPSTF	United States Preventive Services Task Force
WHO	World Health Organization

Scientific Background

Diabetes is a major health concern in the United States. According to the Centers for Disease Control and Prevention :

- Prevalence: In 2021, 38.4 million Americans, or 11.6% of the population, had diabetes. Approximately 1.9 million American children and adults have type 1 diabetes, including about 244,000 children and adolescents.
- Diagnosed and undiagnosed: Of the 38.4 million, 29.7 million were diagnosed, and 8.7 million were undiagnosed.
- Prevalence in seniors: The percentage of Americans aged 65 and older remains high, at 29.2%, or 15.9 million seniors (diagnosed and undiagnosed).
- New cases: 1.2 million Americans are diagnosed with diabetes every year.
- Prediabetes: In 2021, 97.6 million Americans aged eighteen and older had prediabetes.
- Deaths: Diabetes remains the 8th leading cause of death in the United States in 2021, with 103,294 death certificates listing it as the underlying cause of death, and a total of 399.401 death certificates listing diabetes as a cause of death.

- Total economic cost of diabetes care in the United States: $413 billion in 2022 (ADA, 2022; CDC, 2021).

Diabetes can be classified into the following categories:

- "Type 1 diabetes (due to autoimmune β-cell destruction, usually leading to absolute insulin deficiency)"
- "Type 2 diabetes (due to a progressive loss of β-cell insulin secretion frequently on the background of insulin resistance)"
- "Gestational diabetes mellitus (GDM) (diabetes diagnosed in the second or third trimester of pregnancy that was not clearly overt diabetes prior to gestation)"
- "Specific types of diabetes due to other causes, e.g., monogenic diabetes syndromes (such as neonatal diabetes and maturity-onset diabetes of the young [MODY]), diseases of the exocrine pancreas (such as cystic fibrosis and pancreatitis), and drug- or chemical-induced diabetes (such as with glucocorticoid use, in the treatment of HIV/AIDS, or after organ transplantation)" (ElSayed et al., 2023). The diagnosis of diabetes mellitus is easily established when a patient presents with classic symptoms of hyperglycemia, which include polyuria, polydipsia, nocturia, blurred vision, and, infrequently, weight loss. The frequency of symptomatic diabetes has been decreasing in parallel with improved efforts to diagnose diabetes earlier through screening. Increasingly, the majority of patients are asymptomatic, and hyperglycemia is noted on routine laboratory evaluation, prompting further testing (Inzucchi & Lupsa, 2023).

Glycated hemoglobin A1c (also known as HbA1c, A1c, glycohemoglobin, or hemoglobin A1c) testing plays a key role in the management of diabetes. New hemoglobin enters circulation with minimal glucose attached. However, glucose irreversibly binds to hemoglobin based on the surrounding blood glucose concentration. Therefore, A1c is considered a measure of blood glucose level, albeit an indirect one. It is best correlated with the mean glucose level over the last eight to twelve weeks as red blood cells experience significant turnover. Various factors may affect the reliability of A1c (atypical hemoglobins or hemoglobinopathies, chronic kidney disease, et al.), but most assays have been standardized to the Diabetes Control and Complications Trial (DCCT) standard, which "estimated the mean blood glucose concentrations derived from seven measurements a day (before and ninety minutes after each of the three major meals, and before bedtime), performed once every three months and compared the average glucose concentration with A1c values in patients with type 1 diabetes" (Selvin, 2022).

The HbA1c assay provides information about the degree of long-term glucose control (Nathan et al., 1984), and has been recommended for the diagnosis and monitoring of diabetes (ElSayed et al., 2023; IEC, 2009). Various methods of HbA1c measurement include chromatography based HPLC assay, boronate affinity, antibody-based immunoassay, and enzyme based enzymatic assay (Kanyal Butola et al., 2021). Long-term blood sugar control has been associated with decreased risk of retinopathy, nephropathy, neuropathy, and cardiovascular disease, peripheral arterial, cerebrovascular disease (Hanssen et al., 1992) and myocardial fibrosis in adults with diabetes (Al-Badri et al., 2018). Higher HbA1c variability has been associated with higher all-cause mortality in patients with Type 2 Diabetes (Gu et al., 2018).

Fasting plasma glucose is a method of glucose monitoring that measures an individual's glucose level typically in a period defined with no caloric intake for eight hours or more. Its usage in the diagnosis of diabetes lies primarily in gestational diabetes, along with the OGTT, but HbA1c, FPG, or OGTTs with their respective positive results can be used in diagnosing diabetes mellitus in nonpregnant individuals as

well. To diagnose diabetes in asymptomatic individuals, a FPG has to be ≥ 126 mg/dL. For diagnosing prediabetes, an individual may have "impaired fasting glucose," which would present with a range of 100-125 mg/dL (Hayward & Selvin, 2023; Inzucchi & Lupsa, 2023).

Traditionally, the diagnosis of diabetes was predicated on plasma glucose levels as well as symptom presentation. In 2010, the ADA endorsed as a "reliable retrospective marker of blood glucose control over the past 6-8 weeks." The advantages of HbA1c testing include increased convenience, increased stability and decreased variation in measurement. While the ADA 2023 guidelines gave precedence to FPG, the latest 2024 guideline addressed the vital importance of HbA1c for both diagnostic and screening purposes (for both diabetes and prediabetes care).

The ADA notes that there are areas where HbA1c is insufficient and plasma glucose levels are the preferred measurement: "In the presence of hemoglobin variants, pregnancy, glucose-6-phosphate dehydrogenase deficiency, and other conditions that might potentially interfere with accurate HbA1c measurements, plasma glucose levels are preferred. Furthermore, in situations where elevated blood glucose levels might not be consistently apparent, the diagnosis of diabetes necessitates two abnormal test results (HbA1c and plasma glucose) either simultaneously or at different time points. In such scenarios, alternative biomarkers such as fructosamine and glycated albumin emerge as viable options for monitoring glycemic status. Fructosamine reflects the total pool of glycated serum proteins, mainly albumin, reflecting glycemic trends over a span of 2–4 weeks—a relatively shorter duration compared to A1C. Although these biomarkers show a strong correlation and are associated with long-term complications based on epidemiological evidence,the empirical support for their application is not as robust as that for HbA1c" (Tiwari & Aw, 2024).

The OGTT can be more inconvenient and used in the setting to diagnose GDM. Normally, 75g of glucose is ingested by the patient, and if the patient has a two-hour plasma glucose value of ≥200 mg/dL, a diagnosis of diabetes can be made. The test can also be performed at one-hour with 50g oral glucose, with positive GDM diagnostic results between 130-140 mg/dL as part of a two-step approach with the three-hour 100g test, which can be diagnostic of GDM with two elevated values. For prediabetes with an accompanied "impaired glucose tolerance," a two-hour plasma glucose value between 140-199 mg/dL is used. However, the WHO requires an additional FPG <126 in addition to the two-hour plasma glucose value to establish impaired glucose tolerance (Durnwald, 2023; Hayward & Selvin, 2023).

Analytical Validity

The International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) Working Group on HbA1c Standardization has developed a reference measurement system and the measurement of HbA1c is currently well-standardized (Hoelzel et al., 2004), and a sound reference system is in place to ensure continuity and stability of the analytical validity of HbA1c measurement (Weykamp et al., 2008). In contrast, plasma glucose concentration remains difficult to assay with consistent accuracy (Gambino, 2007). HbA1c has greater analytical stability (consistency with repetitive sample testing) and less day-to-day variability than either the fasting plasma glucose (FPG)or two-hour PG (Petersen et al., 2005; Rohlfing et al., 2002). For any given individual, the HbA1c exhibits little short-term biologic variability; its coefficient of variation (CV) is 3.6%, compared to FPG (CV of 5.7%) and 2-h PG (CV of 16.6%) (Malkani & Mordes, 2011; Selvin et al., 2007).

A sample proficiency testing survey performed by the National Glycohemoglobin Standardization Program (NGSP) and College of American Pathologists (CAP) evaluated the accuracy of A1c assays. The survey found that "method-specific, between-laboratory CV's ranged from 0.7% to 4.0%" and

"approximately 85% of laboratories are using methods with CVs <3% at all five HbA1c levels." The survey also noted the current pass limit was ±6%, but using a pass rate of 97.1% to 98.0% of labs passed (NGSP, 2023).

Clinical Utility and Validity

Testing A1c, FPG, and 2-h PG measure different aspects of glycemia and are frequently discordant for diagnosing diabetes. A1c ≥6.5% identifies fewer individuals as having diabetes than glucose-based criteria; however, a recent study concluded that twelve percent of patients can be misclassified with respect to diabetes diagnosis due to laboratory instrument error in measuring glucose (Miller et al., 2008). The New Hoorn Study analyzed the diagnostic properties of the A1c, using OGTT as the diagnostic criterion (van 't Riet et al., 2010). The analysis suggested that an A1c of 5.8% had a sensitivity of 72% and specificity of 91%. This compares with specificity of 24% and sensitivity of 99% for the A1c cut point of 6.5%. On the other hand, the 6.5% cut point had a positive predictive value of 93%, compared with a positive predictive value of only 24% for a cut point of 5.8% (Malkani & Mordes, 2011).

When using the reference diagnosis of diabetes being a two-hour blood glucose >200 mg/dL (11.1 mmol/L) during an OGTT, the specificity of FPG ≥126 mg/dL was >95% and sensitivity about 50%, with possibly lower sensitivities and specificities for individuals over 65 years (Blunt et al., 1991). With the same OGTT reference, the specificity and sensitivity of an A1c ≥6.5%, as per diagnosis of diabetes, were reported as 79% and 44%, respectively (Kramer et al., 2010).

Cowie et al. (2010) "examined prevalence's of previously diagnosed diabetes and undiagnosed diabetes and high risk for diabetes using recently suggested A1c criteria in the U.S. during 2003–2006. We compared these prevalence's to those in earlier surveys and those using glucose criteria." 14,611 individuals were included (completed a household interview) and classified for diagnosed diabetes and by A1c, fasting, and 2-h glucose challenge values. Diagnostic values for A1c were ≥6.5% for "undiagnosed" diabetes and 6%-6.5% for "high risk" of diabetes. The authors found that by these A1c diagnostic values, the "crude prevalence" of diabetes in adults older than twenty years was 20.4 million, of which nineteen percent went undiagnosed based on A1c ≥ 6.5%. The authors then stated that the A1c criteria only diagnosed thirty percent of the undiagnosed diabetic group (Cowie et al., 2010).

Mamtora et al. (2021) assessed the clinical utility of point-of-care (POC) HbA1c testing in the ophthalmology outpatient setting. Forty-nine patients with diabetic retinopathy underwent POC HbA1c testing and blood pressure measurement. Of the 49 patients, 81.6% had POC readings above the recommended HbA1c levels and only 16.3% of these patients were aware of their elevated HbA1c levels. Fourteen patients (33.3%) with high HbA1c readings were referred to secondary diabetic services and 88.8% of patients felt like the test was useful. The authors suggest that POC HbA1c testing is a "cost-effective, reproducible and clinically significant tool for the management of diabetes in an outpatient ophthalmology setting, allowing the rapid recognition of high-risk patients and appropriate referral to secondary diabetic services" (Mamtora et al., 2021).

Goodney et al. (2016) evaluated the consistency of A1c testing of diabetes patients and its effect on cardiovascular outcomes. The study included 1574415 Medicare patients with diabetes mellitus, and the consistency of testing was separated into three categories: "low (testing in zero or one of three years), medium (testing in two of three years), and high (testing in all three years)." Approximately 70.2% of patients received high-consistency testing, 17.6% received medium-consistency, and 12.2% received low-consistency. Major adverse cardiovascular events (MACE) included "death, myocardial infarction, stroke, amputation, or the need for leg revascularization." Low-consistency patients was associated with

death or other adverse events (hazard ratio: 1.21). The authors concluded that "consistent annual hemoglobin A1c testing is associated with fewer adverse cardiovascular outcomes in this observational cohort of Medicare patients of diabetes mellitus" (Goodney et al., 2016).

The GOAL study (Al Mansari et al., 2018) used A1c to assess diabetes control in a real-world practice study aimed to assess predictive factors for achieving the glycemic hemoglobin A1c (HbA1c) at six months as targeted by the treating physician in adults with type 2 diabetes. In this study, 2704 patients with a mean A1c of 9.7% were enrolled. After six months, lower baseline A1c (≥ 8.5% vs <7%) was found to be a predictive factor for achieving glycemic control. The authors also observed "absolute changes in the mean HbA1c of −1.7% and −2% were observed from baseline to six and twelve months, respectively" (Al Mansari et al., 2018).

Mitsios et al. (2018) evaluated the association between A1c and stroke risk. Twenty-nine studies (n=532779) were included. The authors compared the non-diabetic A1c range (<5.7%) to the diabetic range (≥6.5%) and found that the diabetic range was associated with a 2.15-fold increased risk of first-ever stroke. The prediabetes range of 5.7%-6.5% was also not associated with first-ever stroke. The authors also observed that for every one percent increase in A1c, the hazard ratio of first-ever stroke increased (1.12-fold for non-diabetic ranges, 1.17 for diabetic ones). This increased risk was also seen for ischemic stroke, with a hazard ratio of 1.49 for non-diabetic ranges and 1.24 for diabetic ranges (Mitsios et al., 2018).

Ludvigsson et al. (2019) evaluated the association between preterm birth risk and periconceptional HbA1c levels in pregnant individuals with type 1 diabetes (T1D). Preterm birth was defined as <37 weeks and several secondary outcomes were also examined, which were "neonatal death, large-for-gestational age, macrosomia, infant birth injury, hypoglycemia, respiratory distress, five-minute Apgar score less than seven, and stillbirth." A total of 2474 singletons born to individuals with T1D and 1165216 reference infants (children born to mothers without T1D) were included. The authors identified 552 preterm births in the T1D cohort (22.3%) compared to 54287 in the control cohort (4.7%). Incidences of preterm birth were measured at several separate thresholds, including <6.5%, 6.5%-7.8%, 7.8%-9.1%, and >9.1%. The T1D cohort's adjusted risk ratios (aRR) of preterm birth compared to the control cohort were as follows: 2.83 for <6.5%, 4.22 for 6.5%-7.8%, 5.56 for 7.8%-9.1%, and 6.91 for >9.1%. The corresponding aRRs for "medically indicated preterm birth" (n=320) were 5.26, 7.42, 11.75 and 17.51, respectively. Increased HbA1c levels were also found to be associated with the secondary clinical outcomes. The authors concluded that "the risk for preterm birth was strongly linked to periconceptional HbA1c levels (Ludvigsson et al., 2019).

Saito et al. (2019) examined the association of HbA1c variability (defined as visit-to-visit) and later onset of malignancies. The authors included 2640 patients 50 years or older, with diabetes. A total of 330 patients (12.5%) developed malignancies during follow up. The authors stratified the patients into quartiles of glycemic variability (defined as standard deviation of HbA1c) and found a "dose-dependent association with tumorigenesis" in the three highest quartiles. The odds ratios were as follows: 1.20 for the second quartile, 1.43 for the third, and 2.19 for the highest. The authors concluded that "these results demonstrated that visit-to-visit HbA1c variability is a potential risk factor for later tumorigenesis. The association may be mediated by oxidative stress or hormone variability (Saito et al., 2019).

Mañé et al. (2019) evaluated the "suitability of first-trimester fasting plasma glucose and HbA1c levels in non-diabetic range to identify [individuals] without diabetes at increased pregnancy risk." Primary outcomes were defined as "macrosomia and pre-eclampsia" and secondary outcomes were defined as "preterm delivery, Caesarean section and large-for-gestational age." A total of 1228 pregnancies were

included. Pregnant individuals with an HbA1c of ≥5.8% were found to have an increased risk of marcosomia (odds ratio [OR] = 2.69), an HbA1c of ≥5.9% was found to be associated with a three-fold risk of pre-eclampsia, and an HbA1c of ≥6% was found to be associated with a four-fold risk of "large-for-gestational age." FPG levels were not found to be associated with any pregnancy outcome (Mañé et al., 2019).

Arbiol-Roca et al. (2021) studied the clinical utility of HbA1c testing as a biomarker for detecting GDM and as a screening test to avoid the use of the OGTT. HbA1c levels were measured in 745 pregnant individuals and GDM was diagnosed in 38 patients based on HbA1c, age, and BMI. A cut off HbA1c value of 4.6% was determined to decide whether OGTT was needed or if it could be avoided. Using 4.6% HbA1c as the cut off value prevented two false negatives, but only decreased the number of OGTTs performed by 7.2%. The authors conclude that "adoption of HbA1c as a screening test for GDM may eliminate the need of OGTT." Although the HbA1c test does not have sufficient sensitivity and specificity to be used as the sole diagnostic test, " the use of a rule-out strategy in combination with the OGTT could be useful" (Arbiol-Roca et al., 2021).

However, the use of hemoglobin A1c testing is not useful in predicting all forms of dysglycemia. Tommerdahl et al. (2019) evaluated several biomarkers for their accuracy in screening for cystic fibrosis (CF)-related diabetes. These biomarkers included "hemoglobin A1c (HbA1c), 1,5-anhydroglucitol (1,5AG), fructosamine (FA), and glycated albumin (GA)" and were compared to the current gold standard, OGTT 2-hour glucose. Fifty-eight patients with CF were included and "area under the receiver operative characteristic (ROC-AUC) curves were generated." All ROC-AUCs for each biomarker were "low" both for cystic fibrosis-related prediabetes (CFPD, ROC-AUC 0.52-0.67) and CF-related diabetes (CFRD) (0.56-0.61). For CFRD, HbA1c was measured to have a 78% sensitivity and 41% specificity at a cutoff of 5.5%, which corresponds to a ROC-AUC of 0.61. The authors concluded that "All alternate markers tested demonstrate poor diagnostic accuracy for identifying CFRD by 2hG" (Tommerdahl et al., 2019).

In a retrospective review of the UMass Memorial Health System electronic medical records from between 1997 and 2019, Darukhanavala et al. (2021) evaluated the appropriateness of HbA1c as a screening tool for identifying patients with pre-CFRD dysglycemia to minimize the burden of annual two-hour OGTTs. The study included 56 patients categorized according to OGTT results (American Diabetes Association criteria): normal glucose tolerance (n=34), indeterminant glycemia (INDET, n=6), impaired fasting glucose (IFG, n=7), or impaired glucose tolerance (IGT, n=9). It was found that HbA1c was positively correlated with blood glucose levels at the various time cut points (hour zero, hour one, and hour two), though the associations were quite weak ($r = 0.248$, $r = 0.219$, and $r = 0.369$, respectively). Furthermore, t-tests conducted suggested that the mean HbA1c was not significantly different between patients with normal glucose tolerance and those in the INDET ($p = 0.987$), IFG ($p = 0.690$), and IGT ($p = 0.874$) groups, confirmed by ANOVA ($p = 0.250$). Consequently, the authors reported that the "results do not support the use of HbA1c as a possible screening tool for pre-CFRD dysglycemic states, specifically INDET, IFG, and IGT" (Darukhanavala et al., 2021).

By combining administrative datasets from the Veterans Health Administration and Medicare, Zhao et al. (2021) evaluated the impact of hemoglobin A1c (A1c) variability—the CV, described by A1c standard deviation divided by the average A1c value overall and expressed as a percent—on the risk of hypoglycemia-related hospitalization (HRH) in veterans with diabetes mellitus. In this study sample of 342,059 patients, the authors identified a "consistent and positive relationship between A1c variability and HRH" and noted that "Average A1c levels were also significantly and independently associated with HRH, with levels <7.0% (53 mmol/mol) associated with lower risk and levels >9% (75 mmol/mol) conferring greater risk." Due to these different levels of variability all remaining strong predictors of HRH

risk up to three years following the baseline period, authors concluded that "tracking A1c levels alone may be insufficient to mitigate risk." It was also acknowledged that a few limitations affected the generalizability of the study, such as the lack of socioeconomic data, the study sample being predominantly white males, and including only veterans, the latter of which is a population where comorbidities are more prevalent. Consequently, these data may be reflective of "the complex interplay of disease severity, treatment, and sociodemographic factors," as is the case with other clinical findings (Zhao et al., 2021).

While poor outcomes of coronavirus disease 2019 (COVID-19) have been linked to diabetes, its relation to pre-infection glycemic control is still unclear. Because of this, Merzon et al. (2021) investigated the association between pre-infection HemoglobinA1c (A1C) levels and COVID-19 severity as assessed by need for hospitalization in a cohort of 2068 patients (ages 14 to 103) with diabetes tested for COVID-19 in Leumit Health Services, Israel, between February 1 and April 30, 2020. Of the patients in this cohort, 183 (8.85%) were diagnosed with COVID-19. A comparison of the mean HbA1c of those who were COVID-19 positive (7.19%, 95% CI: 6.81%-7.57%) and the mean of those who were COVID-19 negative (6.59%, 95% CI: 6.52%-6.65%) was found to be statistically significant ($p<0.05$). The authors expounded further by reporting the clinical characteristics of patients with diabetes hospitalized due to COVID-19 by demonstrating that the mean Hb1Ac levels between those hospitalized (n=46, 7.75%, 95% CI: 7.17%-8.32%) and those not hospitalized (n=137, 6.83%, 95% CI: 6.54%-7.13%) were also statistically significant ($p<0.005$). Additionally, "In a multivariate logistic regression model adjusting for multiple potential risk factors and chronic conditions which may have a deleterious effect on disease outcomes (including age, sex, smoking, IHD, SES, depression/anxiety, schizophrenia, dementia, hypertension, CVA, CHF, chronic lung disease, and obesity), only HbA1c ≥ nine percent remained a significant predictor for hospitalization." Given the evidence, the researchers urge "Paying special attention to patients with diabetes and an HbA1c ≥ nine while allowing a more lenient approach to patients with well controlled disease," as this can reduce economic, social, and patient burden, especially for those who are at the greatest risk for reacting severely to COVID-19 (Merzon et al., 2021).

Xie et al. (2021) investigated the role of FPG and glucose fluctuation on the prognosis of COVID-19 patients who already had prior diagnoses of diabetes. Through a multivariate Cox analysis, the researchers found that FPG was "an independent prognostic factor of overall survival after adjustment for age, sex, diabetes, and severity of COVID-19 at admission (HR: 1.15, 95% CI: 1.06-1.25)." However, blood glucose fluctuation was associated with COVID-19 disease progression, as proven by the results found from the indices of the standard deviation of blood glucose and the largest amplitude of glycemic excursions. Both FPG and blood glucose fluctuation indices were also found to be positively associated with increased presence of inflammatory markers associated with COVID-19, such as the "white blood cell absolute count, neutrophil count, C-reactive protein, alkaline phosphatase, a-hydroxybutyrate dehydrogenase (α-hbdh), gamma-glutamyl transferase (GGT), lactate dehydrogenase, [and] D-dimer." Ultimately, it was concluded that diabetes was not an independent risk factor for in-hospital death of COVID-19 patients, as these findings were identified regardless of diabetes status (Xie et al., 2021).

Yang et al. (2019) aimed to find the appropriate threshold for FPG for defining prediabetes among children and adolescents. The sample was selected from school-aged children in Taiwan via a nationwide survey administered between 1992-2000, who then underwent physical examinations and blood tests if they exhibited abnormal urine test findings. The researchers found that the incidence of pediatric diabetes increased with increasing fasting plasma glucose levels, and those with FPG > 5.6mmol/L had higher adjusted hazard ratios. Additionally, "the association between fasting plasma glucose and incident pediatric diabetes and the area under the receiver-operating characteristic curve were similar in boys and girls and were higher in the age group twelve to eighteen years." In using 4.75

mmol/L as the optimal threshold for children six to eleven years, the sensitivity was 65% and specificity was 51%. For the threshold of 5.19 mmol/L among children twelve to eighteen years, the sensitivity was 60% and the specificity was 73%. This supports utilizing FPG as a supplement for diagnosing prediabetes among pediatric patients, which may contribute to better disease management.

Geifman-Holtzman et al. (2010) assessed the correlation between fetal macrosomia and abnormal OGTT in pregnant individuals with term gestation and negative glucose challenge test (GCT) at 24 to 28 weeks. They recruited patients who had estimated fetal weights >90th percentile and a negative 50g GCT. From 170 individuals over a five-month period, they found that 10 patients or 5.9% had "impaired glucose metabolism at term." In this group, "we found no correlation between GCT values at twenty-four to twenty-eight weeks, family history of diabetes mellitus, the patient's [body mass index] or weight at term, and the diagnosis of impaired glucose metabolism." Furthermore, there was no statistically significant difference in mean fetal weight between those with normal versus abnormal OGTT. This demonstrated the lack of clinical utility of using OGTT at term for predicting the incidence of fetal macrosomia. The researchers suggested utilizing a larger scale study to solidify or contradict these conclusions (Geifman-Holtzman et al., 2010).

Bi et al. (2024) engaged in a cross-sectional study of participants aged >20 years old who underwent physical examination at the local hospital from 2022 to 2023. A model was used to assess the dose-response relationship between liver enzymes and type 2 diabetes risk. Of the 14,100 participants, an analysis revealed a non-linear relationship between liver enzymes and type 2 diabetes risk (P non-linear < 0.001). Specifically, type 2 diabetes risk increased with rising ALT and GGT levels (range, <50 IU/L) and then leveled out when ALT and GGT levels were >50 IU/L. An elevated AST within a certain range (range, <35 IU/L) decreased the risk of type 2 diabetes, but a mildly elevated AST (>35 IU/L) showed as a risk factor for type 2 diabetes. In conclusion, liver enzymes were associated non-linearly with type 2 diabetes risk in different populations. Higher ALT and GGT levels were shown in this study to increase type 2 diabetes risk as well. In conclusion, additional attention should be paid to elevated liver enzymes and diabetes, but more work also needs to be done to assess association between elevation and T2D risk.

Guidelines and Recommendations

The American Diabetes Association (ADA)

The ADA publishes an extensive guideline encompassing the standards of medical care in diabetes. The 2024 recommendations state:

Classification and Diagnosis of Diabetes (Chapter [Ch] 2) (American Diabetes Association Professional Practice Committee, 2023a):

- Criteria for testing for diabetes or prediabetes in asymptomatic adult:
  - Testing should be considered in overweight or obese (BMI ≥25 kg/m^2 or ≥23 kg/m^2 in Asian Americans) adults who have one or more of the following risk factors:
    - First-degree relative with diabetes
    - High-risk race/ethnicity (e.g., African American, Latino, Native American, Asian American, Pacific Islander)
    - History of CVD
    - Hypertension (≥140/90 mmHg or on therapy for hypertension)
    - HDL cholesterol level <35 mg/dL (0.90 mmol/L) and/or a triglyceride level >250 mg/dL (2.82 mmol/L)

        - Individuals with polycystic ovary syndrome
        - Physical inactivity
        - Other clinical conditions associated with insulin resistance (e.g., severe obesity, acanthosis nigricans)
    - People with prediabetes (A1c ≥5.7% [39 mmol/mol], IGT [impaired glucose tolerance], or IFG [impaired fasting glucose]) should be tested yearly.
    - People who were diagnosed with GDM should have lifelong testing at least every three years.
    - For all other patients, testing should begin at age thirty-five years.
    - If results are normal, testing should be repeated at a minimum of three-year intervals, with consideration of more frequent testing depending on initial results and risk status.
    - People with HIV, exposure to high-risk medicines, history of pancreatitis
- "Diabetes may be diagnosed based on A1C criteria or plasma glucose criteria, either the fasting plasma glucose (FPG) value, 2-h glucose (2-h PG) value during a 75-g oral glucose tolerance test (OGTT), or random glucose value accompanied by classic hyperglycemic symptoms (e.g., polyuria, polydipsia, and unexplained weight loss) or hyperglycemic crises."

A1c

- "The A1C test should be performed using a method that is certified by the National Glycohemoglobin Standardization Program (NGSP) as traceable to the Diabetes Control and Complications Trial (DCCT) reference assay. Grade **B**"
- "Point-of-care A1C testing for diabetes screening and diagnosis should be restricted to U.S. Food and Drug Administration–approved devices at Clinical Laboratory Improvement Amendments (CLIA)–certified laboratories that perform testing of moderate complexity or higher by trained personnel. Grade **B**"
- "Marked discordance between A1C and repeat blood glucose values should raise the possibility of a problem or interference with either test. Grade **B**"
- "In conditions associated with an altered relationship between A1C and glycemia, such as some hemoglobin variants, pregnancy (second and third trimesters and the postpartum period), glucose-6-phosphate dehydrogenase deficiency, HIV, hemodialysis, recent blood loss or transfusion, or erythropoietin therapy, plasma glucose criteria should be used to diagnose diabetes. Grade **B**" **(American Diabetes Association Professional Practice Committee, 2023a; ElSayed et al., 2023)**

Prediabetes and Type 2 Diabetes

- "Screening for prediabetes and type 2 diabetes with an informal assessment of risk factors or validated risk calculator should be done in asymptomatic adults. Grade **B**"
- "Testing for prediabetes and/ or type 2 diabetes in asymptomatic people should be considered in adults of any age with overweight or obesity (BMI ≥25 kg/m^2 or ≥23 kg/m^2 in Asian Americans) who have one or more risk factors. Grade **B**"
- "For all people screening should begin at age thirty-five years. Grade **B**"
- "If tests are normal, repeat screening recommended at a minimum of three-year intervals is reasonable, sooner with symptoms or change in risk (i.e., weight gain). Grade **C**"
- "To screen for prediabetes and type 2 diabetes, fasting plasma glucose, 2-h plasma glucose during 75-g oral glucose tolerance test, and A1C are each appropriate. Grade **B**"
- "When using oral glucose tolerance testing as a screen for diabetes, adequate carbohydrate intake (at least 150 g/ day) should be assured for three days prior to testing. Grade **A**"

- "Risk-based screening for prediabetes and/or type 2 diabetes should be considered after the onset of puberty or after ten years of age, whichever occurs earlier, in children and adolescents with overweight (BMI ≥85th percentile) or obesity (BMI ≥95th percentile) and who have one or more risk factor for diabetes. Grade **B**"
- "Consider screening people for prediabetes or diabetes if on certain medications, such as glucocorticoids, statins, thiazide diuretics, some HIV medications, and second-generation antipsychotic medications, as these agents are known to increase the risk of these conditions. Grade **E**"
- "In people who are prescribed second-generation antipsychotic medications, screen for prediabetes and diabetes at baseline and repeat 12–16 weeks after medication initiation or sooner, if clinically indicated, and annually. Grade **B**"
- "People with HIV should be screened for diabetes and prediabetes with an FPG test before starting antiretroviral therapy, at the time of switching antiretroviral therapy, and 3–6 months after starting or switching antiretroviral therapy. If initial screening results are normal, FPG should be checked annually. Grade **E**"

Cystic Fibrosis-Related Diabetes

- "Annual screening for cystic fibrosis-related diabetes with an oral glucose tolerance test should begin by age ten years in all patients with cystic fibrosis not previously diagnosed with cystic fibrosis-related diabetes. Grade **B**"
- "A1c is not recommended as a screening test for cystic fibrosis–related diabetes due to low sensitivity. However, a value of ≥6.5% (≥48 mmol/mol) is consistent with a diagnosis of CFRD. Grade **B**"
- "Beginning five years after the diagnosis of cystic fibrosis–related diabetes, annual monitoring for complications of diabetes is recommended. Grade **E**"

Gestational Diabetes Mellitus

- "In individuals who are planning pregnancy, screen those with risk factors (**Grade B**) and consider testing all individuals with undiagnosed prediabetes or diabetes (**Grade E**).
- "Before fifteen weeks of gestation, test individuals with risk factors **B** and consider testing all individuals **E** for undiagnosed diabetes at the first prenatal visit using standard diagnostic criteria, if not screened preconception."
- "Before fifteen weeks of gestation, screen for abnormal glucose metabolism to identify individuals who are at higher risk of adverse pregnancy and neonatal outcomes, are more likely to need insulin, and are at high risk of a later gestational diabetes mellitus diagnosis. Grade **B**."
- "Screen for early abnormal glucose metabolism using fasting glucose of 110–125 mg/dL (6.1 mmol/L) or A1C 5.9–6.4% (41–47 mmol/mol). Grade **B**"
- "Screen for gestational diabetes mellitus at twenty-four to twenty-eight weeks of gestation in pregnant individuals not previously found to have diabetes or high-risk abnormal glucose metabolism detected earlier in the current pregnancy. Grade **A**"
- Screen individuals "with gestational diabetes mellitus for prediabetes or diabetes at four to twelve weeks postpartum, using the 75-g oral glucose tolerance test and clinically appropriate nonpregnancy diagnostic criteria. Grade **B**"
- Individuals "with a history of gestational diabetes mellitus should have lifelong screening for the development of diabetes or prediabetes at least every three years. Grade **B**" (ElSayed et al., 2023).

On Diagnostic Tests for Diabetes:

"FPG, 2-h PG during 75-g OGTT, and A1C are appropriate for diagnostic screening. It should be noted that detection rates of different screening tests vary in both populations and individuals. FPG, 2-h PG, and A1C reflect different aspects of glucose metabolism, and diagnostic cut points for the different tests will identify different groups of people. Compared with FPG and A1C cut points, the 2-h PG value diagnoses more people with prediabetes and diabetes" (American Diabetes Association Professional Practice Committee, 2023a).

"The A1C test should be performed using a method that is certified by the National Glycohemoglobin Standardization Program (NGSP) (ngsp.org) and standardized or traceable to the Diabetes Control and Complications Trial (DCCT) reference assay. Point-of-care A1C assays may be NGSP certified and cleared by the U.S. Food and Drug Administration (FDA) for use in monitoring glycemic control in people with diabetes in both Clinical Laboratory Improvement Amendments (CLIA)–regulated and CLIA-waived settings. FDA-approved point-of-care A1C testing can be used in laboratories or sites that are CLIA certified, are inspected, and meet the CLIA quality standards. These standards include specified personnel requirements (including documented annual competency assessments) and participation three times per year in an approved proficiency testing program" (American Diabetes Association Professional Practice Committee, 2023a).

HIV

"People with HIV should be screened for diabetes and prediabetes with an FPG test before starting antiretroviral therapy, at the time of switching antiretroviral therapy, and 3–6 months after starting or switching antiretroviral therapy. If initial screening results are normal, FPG should be checked annually. [**Grade E**] . . . People with HIV are at higher risk for developing prediabetes and diabetes on antiretroviral (ARV) therapies; a screening protocol is therefore recommended. The A1C test may underestimate glycemia in people with HIV; it is not recommended for diagnosis and may present challenges for monitoring" (American Diabetes Association Professional Practice Committee, 2023a).

Glycemic Targets (Ch 6)

- "Assess glycemic status by A1C and/or appropriate continuous glucose monitoring (CGM) metrics at least two times a year. Assess more frequently (e.g., every 3 months) for individuals not meeting treatment goals, with frequent or severe hypoglycemia or hyperglycemia, changing health status, or growth and development in youth." **Grade E**
- "Assess glycemic status at least quarterly and as needed in patients whose therapy has recently changed and/or who are not meeting glycemic goals" **Grade E** (Committee, 2023a).

Children & Adolescents (Ch 14)

The traditional idea of type 2 diabetes occurring only in adults and type 1 diabetes occurring only in children is no longer accurate, as both diseases can occur in both age-groups. The recommendations concerning diabetes testing for children and adolescents are as follows:

- "Risk-based screening for prediabetes and/or type 2 diabetes should be considered after the onset of puberty or ≥10 years of age, whichever occurs earlier, in youth with overweight (BMI ≥85th percentile) or obesity (BMI ≥95th percentile) and who have one or more additional risk

factors for diabetes" (American Diabetes Association Professional Practice Committee, 2023a). Grading based on risk factors;
    - Maternal history of diabetes or GDM during the child's gestation-**Grade A**
    - Family history of type 2 diabetes in first- or second-degree relative-**Grade A**
    - Race/ethnicity (Native American, African American, Latino, Asian American, Pacific Islander)-**Grade A**
    - Signs of insulin resistance or conditions associated with insulin resistance (acanthosis nigricans, hypertension, dyslipidemia, polycystic ovary syndrome, or small-for-gestational-age birth weight)-**Grade B** (American Diabetes Association Professional Practice Committee, 2023a).
- "If tests are normal, repeat screening at a minimum of 3-year intervals [**Grade E**], or more frequently if BMI is increasing [**Grade C**]."
- "Fasting plasma glucose, 2-h plasma glucose during a 75-g oral glucose tolerance test, and A1c can be used to test for prediabetes or [type 2] diabetes in children and adolescents." Grade **B**
- "Children and adolescents with overweight or obesity in whom the diagnosis of type 2 diabetes is being considered should have a panel of pancreatic autoantibodies tested to exclude the possibility of autoimmune type 1 diabetes." **Grade B**
- "Although A1c is not recommended for diagnosis of diabetes in children with cystic fibrosis or symptoms suggestive of acute onset of type 1 diabetes and only A1c assays without interference are appropriate for children with hemoglobinopathies, ADA continues to recommend A1c for diagnosis of type 2 diabetes in this population (ungraded)"
- "A1C goals must be individualized and reassessed over time. An A1C of <7% (53 mmol/mol) is appropriate for many children" **Grade B** (Committee, 2023b).

Pregnancy (Ch 15)

- "…although A1c may be useful, it should be used as a secondary measure of glycemic control in pregnancy, after blood glucose monitoring."
- "Fasting, preprandial, and postprandial blood glucose monitoring are recommended in individuals with diabetes in pregnancy to achieve optimal glucose levels. Glucose goals are fasting plasma glucose <95 mg/dL (<5.3 mmol/L) and either 1-h postprandial glucose <140 mg/dL (<7.8 mmol/L) or 2-h postprandial glucose <120 mg/dL (<6.7 mmol/L)Grade **B**"
- "Due to increased red blood cell turnover, A1C is slightly lower during pregnancy in people with and without diabetes. Ideally, the A1C goal in pregnancy is <6% (<42 mmol/mol) if this can be achieved without significant hypoglycemia, but the goal may be relaxed to <7% (<53 mmol/mol) if necessary to prevent hypoglycemia Grade **B"**
- "Given the alteration in red blood cell kinetics during pregnancy and physiological changes in glycemic parameters, A1c levels may need "to be monitored more frequently than usual (e.g., monthly)."
- "The OGTT is recommended over A1C at four to twelve weeks postpartum because A1C may be persistently impacted (lowered) by the increased red blood cell turnover related to pregnancy, by blood loss at delivery, or by the preceding three-month glucose profile. The OGTT is more sensitive at detecting glucose intolerance, including both prediabetes and diabetes."
- "Because GDM often represents previously undiagnosed prediabetes, type 2 diabetes, maturity-onset diabetes of the young, or even developing type 1 diabetes, individuals with GDM should be tested for persistent diabetes or prediabetes at four to twelve weeks postpartum with a fasting 75-g OGTT using nonpregnancy criteria as outlined in Section two, "Classification and Diagnosis of Diabetes."

- "In the absence of unequivocal hyperglycemia, a positive screen for diabetes requires two abnormal values. If both the fasting plasma glucose (≥126 mg/dL [7.0 mmol/L]) and 2-h plasma glucose (≥200 mg/dL [11.1 mmol/L]) are abnormal in a single screening test, then the diagnosis of diabetes is made. If only one abnormal value in the OGTT meets diabetes criteria, the test should be repeated to confirm that the abnormality persists."
- "Individuals with a history of GDM should have ongoing screening for prediabetes or type 2 diabetes every 1–3 years, even if the results of the initial 4–12 week postpartum 75-g OGTT are normal. Ongoing evaluation may be performed with any recommended glycemic test (e.g., annual A1C, annual fasting plasma glucose, or triennial 75-g OGTT using thresholds for nonpregnant individuals)" (American Diabetes Association Professional Practice Committee, 2023e).

Heart Failure Considerations (ch. 10)

- "In asymptomatic individuals, routine screening for coronary artery disease is not recommended, as it does not improve outcomes as long as ASCVD risk factors are treated." Grade A
- "Consider investigations for coronary artery disease in the presence of any of the following: atypical cardiac symptoms; signs or symptoms of associated vascular disease, including carotid bruits, transient ischemic attack, stroke, claudication, or PAD; or electrocardiogram abnormalities (e.g., Q waves)." Grade E
- "Adults with diabetes are at increased risk for the development of asymptomatic cardiac structural or functional abnormalities (stage B heart failure) or symptomatic (stage C) heart failure. Consider screening adults with diabetes by measuring a natriuretic peptide (B-type natriuretic peptide [BNP] or N-terminal pro-BNP [NTproBNP]) to facilitate prevention of stage C heart failure." Grade B
- "In asymptomatic individuals with diabetes and abnormal natriuretic peptide levels, echocardiography is recommended to identify stage B heart failure." Grade A
- "In asymptomatic individuals with diabetes and age ≥50 years, microvascular disease in any location, or foot complications or any end-organ damage from diabetes, screening for PAD with ankle-brachial index testing is recommended to guide treatment for cardiovascular disease prevention and limb preservation. A In individuals with diabetes duration ≥10 years, screening for PAD should be considered" Grade B (American Diabetes Association Professional Practice Committee, 2023c).

Nonalcoholic Fatty Liver Disease and Nonalcoholic Steatohepatitis & Chronic Kidney Disease (ch. 4 and ch. 11)

From chapter 4:

- "Adults with type 2 diabetes or prediabetes, particularly those with obesity or cardiometabolic risk factors or established cardiovascular disease, should be screened/risk stratified for clinically significant liver fibrosis (defined as moderate fibrosis to cirrhosis) using a calculated fibrosis-4 index (FIB-4) (derived from age, ALT, AST, and platelets…even if they have normal liver enzymes." Grade **B**
- "Adults with diabetes or prediabetes with persistently elevated plasma aminotransferase levels for >6 months and low FIB-4 should be evaluated for other causes of liver disease." Grade B
- "Adults with type 2 diabetes or prediabetes with an indeterminate or high FIB-4 should have additional risk stratification by liver stiffness measurement with transient elastography or the blood biomarker enhanced liver fibrosis (ELF)." Grade **B**

- "Adults with type 2 diabetes or prediabetes with indeterminate results or at high risk for significant liver fibrosis (i.e., by FIB-4, liver stiffness measurement, or ELF) should be referred to a gastroenterologist or hepatologist for further workup. Interprofessional care is recommended for long-term management Grade **B**" (American Diabetes Association Professional Practice Committee, 2023b).(American Diabetes Association Professional Practice, 2022)

From chapter 11:

Additionally: "A screening strategy based on elevated plasma aminotransferases >40 units/L would miss most individuals with NASH in these settings, as clinically significant fibrosis (≥F2) is frequently observed with plasma aminotransferases below the commonly used cutoff of 40 units/L. The American College of Gastroenterology considers the upper limit of normal ALT levels to be 29–33 units/L for male individuals and 19–25 units/L for female individuals, as higher levels are associated with increased liver-related mortality, even in the absence of identifiable risk factors. The FIB-4 estimates the risk of hepatic cirrhosis and is calculated from the computation of age, plasma aminotransferases (AST and ALT), and platelet count"(American Diabetes Association Professional Practice Committee, 2023d).

In regards to A1c and NASH, the ADA restricts its comments to the following: "The only proven primary prevention interventions for CKD in people with diabetes are blood glucose (A1C goal of 7%) and blood pressure control (blood pressure <130/80 mmHg)," and "Intensive lowering of blood glucose with the goal of achieving near-normoglycemia has been shown in large, randomized studies to delay the onset and progression of albuminuria and reduce eGFR in people with type 1 diabetes and type 2 diabetes. Insulin alone was used to lower blood glucose in the Diabetes Control and Complications Trial (DCCT)/Epidemiology of Diabetes Interventions and Complications study of type 1 diabetes, while a variety of agents were used in clinical trials of type 2 diabetes, supporting the conclusion that lowering blood glucose itself helps prevent CKD and its progression. The effects of glucose-lowering therapies on CKD have helped define A1C goals" (American Diabetes Association Professional Practice Committee, 2023d).

Hospital Care Delivery Standards and Perioperative Care (ch. 16)

- "Perform an A1C test on all people with diabetes or hyperglycemia (random blood glucose >140 mg/dL [>7.8 mmol/L]) admitted to the hospital if no A1C test result is available from the prior 3 months." Grade B
- "In hospitalized individuals with diabetes who are eating, point-of-care (POC) blood glucose monitoring should be performed before meals; in those not eating, glucose monitoring is advised every 4–6 h. More frequent POC blood glucose monitoring ranging from every 30 min to every 2 h is the required standard for safe use of intravenous insulin therapy." (No grade; statement)

The following approach may be considered for those in preoperative and perioperative care:

- "A preoperative risk assessment should be performed for people with diabetes who are at high risk for ischemic heart disease and those with autonomic neuropathy or renal failure.
- The A1C goal for elective surgeries should be <8% (<63.9 mmol/L) whenever possible.
- The blood glucose goal in the perioperative period should be 100–180 mg/dL (5.6–10.0 mmol/L) within 4 h of the surgery. CGM should not be used alone for glucose monitoring during surgery.
- Metformin should be held on the day of surgery.
- SGLT2 inhibitors should be discontinued 3–4 days before surgery.

- Hold other oral glucose-lowering agents the morning of surgery or procedure and give one-half of NPH dose or 75–80% doses of long-acting analog insulin or adjust insulin pump basal rates based on the type of diabetes and clinical judgment.
- Monitor blood glucose at least every 2–4 h while the individual takes nothing by mouth and dose with short- or rapid-acting insulin as needed.
- There are little data on the safe use and/or influence of GLP-1 receptor agonists on glycemia and delayed gastric emptying in the perioperative period.
- Stricter perioperative glycemic goals are not advised, as perioperative glycemic goals stricter than 80–180 mg/dL (4.4–10.0 mmol/L) may not improve outcomes and are associated with more hypoglycemia.
- Compared with usual dosing, a reduction by 25% of basal insulin given the evening before surgery is more likely to achieve perioperative blood glucose goals with a lower risk for hypoglycemia.
- In individuals undergoing noncardiac general surgery, basal insulin plus premeal short- or rapid-acting insulin (basal-bolus) coverage has been associated with improved glycemic outcomes and lower rates of perioperative complications compared with the reactive, correction-only short- or rapid-acting insulin coverage alone with no basal insulin dosing." (American Diabetes Association Professional Practice Committee, 2023f).

The ADA did not specifically mention "bariatric surgery" in their hospital care delivery section (ch. 16).

Diabetes Canada Clinical Practice Guidelines Expert Committee

This Expert Committee published a comprehensive guideline on the prevention and management of diabetes. Relevant items, recommendations, and comments—particularly those relating to the use of A1c testing—are captured below:

- "Screen for type 2 diabetes using a fasting plasma glucose and/or glycated hemoglobin (A1C) every three years in individuals ≥40 years of age or in individuals at high risk on a risk calculator (33% chance of developing diabetes over ten years)."
- "In the absence of evidence for interventions to prevent or delay type 1 diabetes, routine screening for type 1 diabetes is not recommended."
- "For most individuals with diabetes, A1C should be measured approximately every three months to ensure that glycemic goals are being met or maintained. In some circumstances, such as when significant changes are made to therapy, or during pregnancy, it is appropriate to check A1C more frequently. Testing at least every six months should be performed in adults during periods of treatment and healthy behavior stability when glycemic targets have been consistently achieved."
- A1C can be misleading in various medical conditions ("e.g., hemoglobinopathies, iron deficiency, hemolytic anemia, severe hepatic or renal disease") and should not be used for "diagnostic use in children and adolescents (as the sole diagnostic test), pregnant [individuals] as part of routine screening for gestational diabetes, those with cystic fibrosis or those with suspected type 1 diabetes."
- Diabetes "should" be diagnosed at a level of A1C ≥6.5%.
- "Screening for diabetes using FPG and/or A1C should be performed every three years in individuals ≥40 years of age or at high risk using a risk calculator [Grade D, Consensus]. Earlier testing and/or more frequent follow up (every six to twelve months) with either FPG and/or A1C should be considered in those at very high risk using a risk calculator or in people with additional risk factors for diabetes [Grade D, Consensus]"

It should be mentioned that "Glycemic targets should be individualized [Grade D, Consensus]" based upon various considerations including, but not limited to, the patient's functional dependence, medical history, life expectancy, and life course stage. Moreover, the grading of recommendations above (e.g., "Grade D") reflect the methodological rigor used at arriving at the conclusion, such that lower grades reflect the presence of weaker evidence. But though the "paucity of clinical evidence addressing the areas of therapy, prevention, diagnosis or prognosis precluded the assignment of a higher grade," the authors recognize and note that many Grade D recommendations are "very important to the contemporary management of diabetes" (Diabetes Canada Clinical Practice Guidelines Expert Committee, 2018).

The United States Preventive Services Task Force (USPSTF)

The USPSTF recommends screening for prediabetes and type 2 diabetes in adults aged 35 to 70 years who are overweight or obese, and such "Screening tests for prediabetes and type 2 diabetes include measurement of fasting plasma glucose or HbA1c level or an oral glucose tolerance test." Recognizing that "The optimal screening interval for adults with an initial normal glucose test result is uncertain," the USPSTF suggests that "Screening every three years may be a reasonable approach for adults with normal blood glucose levels" (Davidson et al., 2021).

The USPSTF has also provided guidelines pertaining to the screening of gestational diabetes. For asymptomatic pregnant persons at 24 weeks gestation or after, with a letter "B" grade, the USPSTF recommends screening for gestational diabetes in this population. However, in asymptomatic pregnant persons before 24 weeks gestation, the USPSTF states that "current evidence is insufficient to assess the balance of benefits and harms of screening" and has given it an "I" grade (USPSTF, 2021). An "I" grade is defined by the USPSTF as "I Statement- The USPSTF concludes that the current evidence is insufficient to assess the balance of benefits and harms of the service. Evidence is lacking, of poor quality, or conflicting, and the balance of benefits and harms cannot be determined"(USPSTF, 2018).

In 2022, the USPSTF released its first recommendation on screening for type 2 diabetes in children and adolescents. This recommendation applies to children and adolescents who are not pregnant and who are younger than 18 years of age without known diabetes or prediabetes and who are without symptoms of diabetes or prediabetes. The USPSTF states that the goal of screening for type 2 diabetes in young people is "to diagnose and treat it early to prevent development of bad health outcomes. However, no studies have looked at the link between screening for type 2 diabetes in children and adolescents and bad health outcomes. Studies about the effect of type 2 diabetes treatment on health outcomes in children and adolescents have not had enough patients with bad outcomes to draw any meaningful conclusions. No studies have looked at harms of screening for type 2 diabetes in young people. Potential harms may include side effects from medications used to treat diabetes, such as low blood glucose, nausea, or vomiting." Based on the current evidence for asymptomatic children and adolescents younger than 18 years of age, the USPSTF concluded that "current evidence is insufficient to assess the balance of benefits and harms of screening for type 2 diabetes in children and adolescents" and has given it an "I" grade (Jin, 2022).

World Health Organization (WHO)

The Global Report on Diabetes (WHO, 2016) states that: "Glycated haemoglobin (HbA1c) is the method of choice for monitoring glycaemic control in diabetes. An advantage of using HbA1c is that the patient does not need to be in a fasting state. Ideally it should be measured twice a year in people with type 2 diabetes and more frequently in those with type 1 diabetes. However, HbA1c testing is more costly than

glucose measurement, and therefore less readily available. If HbA1c testing is not available, fasting, or post-meal blood glucose is an acceptable substitute."

The WHO also published a "module" titled "Hearts-D: Diagnosis and Management of Type 2 Diabetes in 2020. In it, a testing algorithm for "treatment of type 2 diabetes mellitus with insulin" is included at the bottom. The algorithm calls for an HbA1c assessment to be performed "in three months" if the patient is stabilized as a result of the insulin treatment (WHO, 2020).

American Academy of Family Physicians (AAFP)

In 2022, the AAFP published a clinical summary of the USPSTF recommendation for screening for prediabetes and type 2 diabetes mellitus. The document deferred to the USPSTF recommendations, with the testing audience being "Nonpregnant adults aged thirty-five to seventy years who have overweight or obesity and no symptoms of diabetes"—a move from 40 years of age in the previous recommendation—while deeming screening every three years to be a reasonable approach (AAFP, 2022).

Endocrine Society

The Endocrine Society published this guideline regarding management of diabetes in older adults. In it, they recommend screening for prediabetes or diabetes every two years for patients 65 years or older. FPG and/or HbA1c may be used. However, the Society does recommend caution when interpreting HbA1c results, as older patients are more likely to have conditions that alter red blood cell turnover (LeRoith et al., 2019).

National Institute for Health and Care Excellence (NICE)

NICE published an update to their guideline on diabetes management. In it, they make the following recommendations:

"Measure HbA1c levels in adults with type 2 diabetes every:
- Three to six months (tailored to individual needs) until HbA1c is stable on unchanging therapy.
- Six months once the HbA1c level and blood glucose lowering therapy are stable."

"Measure HbA1c using methods calibrated according to International Federation of Clinical Chemistry (IFCC) standardization."

"If HbA1c monitoring is invalid because of disturbed erythrocyte turnover or abnormal haemoglobin type, estimate trends in blood glucose control using one of the following:
- quality-controlled plasma glucose profiles
- total glycated haemoglobin estimation (if abnormal haemoglobins)
- fructosamine estimation."

"Investigate unexplained discrepancies between HbA1c and other glucose measurements. Seek advice from a team with specialist expertise in diabetes or clinical biochemistry." (NICE, 2022)

American Association of Clinical Endocrinologists (AACE)

The AACE provides the following inclusion criteria for individuals who should be screened for prediabetes or type 2 diabetes:

- Age ≥45 years without other risk factors
- CVD or family history of T2D
- Overweight or obese
- Sedentary lifestyle
- Member of an at-risk racial or ethnic group:
  - Asian
  - African American
  - Hispanic
  - Native American (Alaska Natives and American Indians)
  - Pacific Islander
- High-density lipoprotein cholesterol (HDL-C) <35 mg/dL (0.90 mmol/L) and/or a triglyceride level >250 mg/dL (2.82 mmol/L)
- Impaired glucose tolerance (IGT), impaired fasting glucose (IFG), and/or metabolic syndrome
- Polycystic ovary syndrome (PCOS), acanthosis nigricans, or nonalcoholic fatty liver disease (NAFLD)
- Hypertension (blood pressure >140/90 mm Hg or on antihypertensive therapy)
- History of gestational diabetes or delivery of a baby weighing more than 5 kg (9 lb)
- Antipsychotic therapy for schizophrenia and/or severe bipolar disease
- Chronic glucocorticoid exposure
- Sleep disorders in the presence of glucose intolerance (A1C >5.7%, IGT, or IFG on previous testing), including obstructive sleep apnea (OSA), chronic sleep deprivation, and night-shift occupation

The AACE recommends repeat testing at least every three years for individuals with normal results. Consider annual screening for patients with two or more risk factors.

In a 2022 update focusing on developing a diabetes mellitus comprehensive care plan, the AACE expounds on how the diagnosis of diabetes mellitus should be made. According to the authors, the ELs refer to evidence levels established by AACE evidence ratings, where "descriptors of "must," "should," and "may" generally but not strictly correlate with Grade A (strong), Grade B (intermediate), and Grade C (weak) recommendations, respectively" (Blonde et al., 2022). The relevant recommendations are captured below.

"*Recommendation 1.1*

The diagnosis of DM is based on the following criteria…:

- FPG concentration ≥126 mg/dL (after ≥ eight hours of an overnight fast), or
- Plasma glucose (PG) concentration ≥200 mg/dL two hours after ingesting a 75-g oral glucose load after an overnight fast of at least eight hours, or
- Symptoms of hyperglycemia (e.g., polyuria, polydipsia, polyphagia) and a random (nonfasting) PG concentration ≥200 mg/dL, or
- A1C level ≥6.5%

Diagnosis of DM requires two abnormal test results, either from the same sample or two abnormal results on samples drawn on different days. However, a glucose level ≥200 mg/dL in the presence of symptoms for DM confirms the diagnosis of DM.

Grade A; BEL 2 and expert opinion of task force

Recommendation 1.2

Prediabetes is identified by the presence of IFG (100 to 125 mg/dL), impaired glucose tolerance (IGT), which is a PG value of 140 to 199 mg/dL two hours after ingesting 75 g of glucose, and/or A1C value between 5.7% and 6.4% (Table 4). A1C should be used only for screening for prediabetes. The diagnosis of prediabetes, which may manifest as either IFG or IGT, should be confirmed with glucose testing.

Grade B; BEL 2

Recommendation 1.3

T1D is characterized by marked insulin deficiency in the presence of hyperglycemia and positive autoantibody tests to glutamic acid decarboxylase (GAD65), pancreatic islet β cells (tyrosine phosphatase IA-2), and IA-2b zinc transporter (ZnT8), and/or insulin. The presence of immune markers and clinical presentation are needed to establish the correct diagnosis and to distinguish between T1D and T2D in children or adults, as well as to determine appropriate treatment.

Grade A; BEL 2

Recommendation 1.4

T2D is characterized by progressive loss of β-cell insulin secretion and variable defects in insulin sensitivity. T2D is often asymptomatic and can remain undiagnosed for many years; therefore, all adults ≥35 years of age with risk factors should be screened for DM (Table 5).

Grade A; BEL 1

Recommendation 1.5

GDM is defined as carbohydrate intolerance that begins or is first recognized during pregnancy and resolves postpartum. Pregnant individuals with risk factors for DM should be screened at the first prenatal visit for undiagnosed T2D using standard criteria (Table 4).

Grade B; BEL 1

Recommendation 1.6

Screen all pregnant individuals for GDM at twenty-four to twenty-eight weeks' gestation. Diagnose GDM with either the one-step or the two-step approach.

- The one-step approach uses a two-hour 75-g oral glucose tolerance test (OGTT) after ≥ eight hours of fasting with diagnostic cutoffs of one or more FPG ≥92 mg/dL, one-hour PG ≥180 mg/dL, or two-hour PG ≥153 mg/dL.
- The two-step approach uses a nonfasting one-hour 50-g glucose challenge test with one-hour PG screening threshold of 130 or 140 mg/dL. For individuals with a positive screening test, the three-hour 100-g OGTT is used for diagnosis with two or more PG tests that meet the following thresholds: FPG ≥95 mg/dL, 1-hour ≥180 mg/dL, 2-hour ≥155 mg/dL, 3-hour ≥140 mg/dL.

Grade A; BEL 1

Recommendation 1.7

Clinicians should consider evaluation for monogenic DM in any child or young adult with an atypical presentation, clinical course, or response to therapy. Monogenic DM includes neonatal diabetes and nonautoimmune diabetes of multiple genetic causes, also known as maturity-onset diabetes of the young (MODY). Most children with DM occurring under six months of age have a monogenic cause as autoimmune T1D rarely occurs before six months of age. Other monogenic forms of diabetes are characterized by mutation of genes of transcription factors, genes regulating pancreatic development or atrophy, abnormal insulin genes, genes related to endoplasmic reticulum stress that impair insulin secretion, or abnormal glucokinase genes that cause impaired insulin signaling.

Grade B; BEL 2

Although not expressly listed as recommendations for diabetes screening, some additional information of note includes the following:

- "A glucose level ≥200 mg/dL in the presence of hyperglycemia symptoms such as polyuria and polydipsia confirm the diagnosis of DM. In individuals with discordant results from two different tests, the test result that is above the diagnostic cut point should be repeated on a different day."
- "In view of physiological changes in pregnancy that could affect glycated hemoglobin levels, A1C should not be used for GDM screening or diagnosis of DM."
- "All pregnant individuals should be screened for GDM at twenty-four to twenty-eight weeks' gestation. Universal screening is recommended, as selective screening (only in individuals with risk factors) would miss a significant number of individuals with GDM and universal screening has been shown to be cost-effective compared with selective screening" (Blonde et al., 2022).

American Association of Clinical Endocrinologists/American College of Endocrinology (AACE/ACE)

The 2020 Consensus Statement from the AACE/ACE on the Management of Type 2 Diabetes states:

- "The hemoglobin A1c (A1c) target should be individualized based on numerous factors such as age, life expectancy, comorbid conditions, duration of diabetes, risk of hypoglycemia or adverse consequences from hypoglycemia, patient motivation, and adherence."
- "An A1c level of ≤6.5% is considered optimal if it can be achieved in a safe and affordable manner, but higher targets may be appropriate for certain individuals and may change for a given individual over time."
- "Therapy must be evaluated frequently (e.g., every three months) until stable using multiple criteria, including A1c, SMBG records (fasting and postprandial) or continuous glucose monitoring tracings, documented and suspected hypoglycemia events, lipid and BP values, adverse events (weight gain, fluid retention, hepatic or renal impairment, or CVD), comorbidities, other relevant laboratory data, concomitant drug administration, complications of diabetes, and psychosocial factors affecting patient care. Less frequent monitoring is acceptable once targets are achieved" (Garber et al., 2020).

In 2023, the AACE/ACE released "Guidelines and Recommendations for Laboratory Analysis in the Diagnosis and Management of Diabetes Mellitus" (Sacks et al., 2023).

Diagnosis related recommendations:

- "Fasting glucose should be measured in venous plasma when used to establish the diagnosis of diabetes, with a value ≥7.0 mmol/L (≥126 mg/dL) diagnostic of diabetes. A (high)"

Screening related recommendations:

- "Recommendation: Screening by HbA1c, FPG, or 2-h OGTT is recommended for individuals who are at high risk of diabetes. If HbA1c is <5.7% (<39 mmol/mol), FPG is <5.6 mmol/L (<100 mg/dL), and/or 2-h plasma glucose is <7.8 mmol/L (<140 mg/dL), testing should be repeated at 3-year intervals. B (moderate)
- Recommendation: Glucose should be measured in venous plasma when used for screening of high-risk individuals. B (moderate)
- Recommendation: Plasma glucose should be measured in an accredited laboratory when used for diagnosis of or screening for diabetes. GPP (good practice point)"

Monitoring/Prognosis:

- "Recommendation: Routine measurement of plasma glucose concentrations in a laboratory is not recommended as the primary means of monitoring or evaluating therapy in individuals with diabetes. B (moderate)"

Kidney Disease: Improving Global Outcomes (KDIGO) Diabetes Working Group

The KDIGO group published recommendations on diabetes and chronic kidney disease (CKD). They recommend using HbA1c to monitor diabetic and CKD patients twice a year or as often as four times a year if glycemic target is not met or a change is made in therapy. KDIGO advises that "accuracy and precision of HbA1c measurement declines with advanced CKD, particularly among patients treated by dialysis, in whom HbA1c measurements have low reliability." They also recommend an "individualized HbA1c target ranging from <6.5% to <8.0% in patients with diabetes and CKD not treated with dialysis" (Rossing et al., 2022).

American College of Gastroenterology

Metabolic dysfunction-associated steatotic liver disease (MASLD) is a condition where there is a buildup of fat in the liver. It is seen in individuals who drink little to no alcohol but who have diabetes, obesity, high blood pressure, or high cholesterol. Diabetes is both a possible cause of and or symptom of MASLD: while diabetes is a risk factor for developing MASLD, individuals who have been diagnosed with MASLD may be at risk for developing heart disease and diabetes (ACG, 2024).

Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
82947	Glucose; quantitative, blood (except reagent strip)
82951	Glucose; tolerance test (GTT), 3 specimens (includes glucose)
82952	Glucose; tolerance test, each additional beyond 3 specimens
82985	Glycated protein
83036	Hemoglobin; glycosylated (A1C)
83037	Hemoglobin; glycosylated (A1C) by device cleared by FDA for home use

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

Evidence-based Scientific References

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Revision History

Revision Date	Summary of Changes
12/04/2024	Reviewed and Updated: Updated background, guidelines, and evidence-based scientific references. Literature review necessitated the following changes in coverage criteria: Addition of CC2e: "e) Quarterly for individuals who are pregnant. Addition of CC5i: "i) For individuals with metabolic dysfunction-associated steatotic liver disease (MASLD)." Removed Note 1, support for testing is found in the guidelines section of policy documents. "Note 1: According to the American Diabetes Association (ADA), measurement of plasma glucose is sufficient to diagnose diabetes mellitus in a patient with classic symptoms (polyuria, polyphagia, polydipsia)." Results in changing note numbering and references within criteria.

Diagnosis of Idiopathic Environmental Intolerance

Policy Number: AHS – G2056 – Diagnosis of Idiopathic Environmental Intolerance	Initial Presentation Date: 09/18/2015 Effective date: 4/1/2025

Policy Description

Idiopathic environmental intolerance (IEI), formerly called multiple chemical sensitivity (MCS), is a subjective condition characterized by recurrent, nonspecific symptoms attributed to low levels of chemical, biologic, or physical agents in the absence of consistent objective diagnostic physical findings or laboratory tests that define an illness (AAAAI, 1999; ACOEM, 1999; Black & Temple, 2024).

Related Policies

Policy Number	Policy Title
AHS-G2031	Allergen Testing
AHS-G2099	Intracellular Micronutrient Analysis

Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

1) In all circumstances, laboratory tests designed to confirm the diagnosis of idiopathic environmental intolerance **DO NOT MEET COVERAGE CRITERIA**.

2) In all circumstances, the screening of blood, saliva, serum, plasma, urine, and/or stool samples for volatile solvents, organic acids, and organophosphates **DOES NOT MEET COVERAGE CRITERIA**.

3) In all circumstances, profiling of phthalates and parabens using a blood, serum, plasma, saliva, urine, and/or stool sample **DOES NOT MEET COVERAGE CRITERIA**.

4) For asymptomatic individuals, profiling of chlorinated pesticides, including DDE and DDT, using a blood, serum, plasma, saliva, urine, and/or stool sample **DOES NOT MEET COVERAGE CRITERIA**.

5) In asymptomatic individuals and/or during general encounters without abnormal findings, testing of blood, serum, plasma, saliva, urine, and/or stool samples for carnitine sufficiency, oxidative stress and antioxidant sufficiency, detoxification adequacy, methylation sufficiency status, lipoic acid and CoQ10 sufficiency, and/or intestinal hyperpermeability **DO NOT MEET COVERAGE CRITERIA**.

6) In asymptomatic individuals and/or during general encounters without abnormal findings, testing of blood, serum, plasma, saliva, urine, and/or stool samples for vitamin sufficiency, mineral sufficiency, and/or nutritional analysis **DO NOT MEET COVERAGE CRITERIA**.

7) The use of a breath hydrogen and/or breath methane test to assess or diagnose the following conditions **DOES NOT MEET COVERAGE CRITERIA**:
    a) Idiopathic environmental intolerance.
    b) Food allergies and sensitivities.
    c) Carbohydrate sensitivity or intolerance.
    d) Bacterial overgrowth, including but not limited to, small intestinal bacterial overgrowth [SIBO].
    e) Digestive disorders.
    f) Constipation, diarrhea, or flatulence.
    g) Neurological/neuromuscular disorders.
    h) Rosacea.
    i) Obesity.
    j) As part of a wellness visit and/or general encounter without abnormal findings.

8) In asymptomatic individuals and/or during general encounters without abnormal findings, testing of blood, serum, urine, cerebrospinal fluid, fingernails, hair, and/or stool sample for metals **DOES NOT MEET COVERAGE CRITERIA**.

Reimbursement Policy

1) For 83918 (Organic acids; total, quantitative, each specimen), a maximum of 2 units per date of service is **ALLOWED**.
2) For 83919 (Organic acids; qualitative, each specimen), a maximum of 1 unit per date of service is **ALLOWED**.
3) For 83921 (Organic acid, single, quantitative), a maximum of 2 units per date of service is **ALLOWED**.
4) For 82127 (Amino acids; single, qualitative, each specimen), a maximum of 1 unit per date of service is **ALLOWED**.
5) For 82136 (Amino acids, 2 to 5 amino acids, quantitative, each specimen), a maximum of 2 units per date of service is **ALLOWED**.
6) For 82139 (Amino acids, 6 or more amino acids, quantitative, each specimen), a maximum of 2 units per date of service is **ALLOWED**.
7) For 84585 (Vanillylmandelic acid (VMA), urine), a maximum of 1 unit per date of service is **ALLOWED**.
8) For 83150 (Homovanillic acid (HVA)), a maximum of 1 unit per date of service is **ALLOWED**.
9) For 83497 (Hydroxyindolacetic acid, 5-(HIAA)), a maximum of 1 unit per date of service is **ALLOWED**.
10) For 82656 (Elastase, pancreatic (EL-1), fecal, qualitative or semi-quantitative), a maximum of 1 unit per date of service is **ALLOWED**.

Table of Terminology

Term	Definition
5-HIAA	5-hydroxyindolacetic acid
AAAAI	American College of Physicians and the American Academy of Allergy and Immunology
ACOEM	American College of Occupational and Environmental Medicine
ACP	American College of Physicians
AMA	American Medical Association
ANA	Antinuclear antibodies
AND	The Academy of Nutrition and Dietetics
ANSES	French Agency for Food, Environmental and Occupational Health & Safety
BPA	Bisphenol A
BT	Breath test
CDSA	Comprehensive digestive stool analysis
CLIA '88	Clinical Laboratory Improvement Amendments of 1988
CH4	Methane
CMS	Centers for Medicare and Medicaid Services
CoQ10	Coenzyme Q10/ubiquinone-10
DAO	Enzyme diamine oxidase
DDE	Dichlorodiphenyldichloroethylene
DDT	Dichlorodiphenyltrichloroethane
DEDTP	Diethydithiophosphate

DETP	Diethylthiophosphate
DMDTP	Dimethyldithiophosphate
DMTP	Dimethylthiophosphate
DNMCC	Does not meet coverage criteria
EESI	Environmental exposure and sensitivity intolerance
EHS	Electromagnetic hypersensitivity
EL-1	Elastase (pancreatic)
ESPGHAN	European Society for Pediatric Gastroenterology, Hepatology, and Nutrition
FDA	Food and Drug Administration
FMV®	First morning void
GC	Gas chromatography
GHBT	Glucose hydrogen breath tests
GI	Gastrointestinal
HPLC	High performance liquid chromatography
HVA	Homovanillic acid
H2	Hydrogen
IBS	Irritable bowel syndrome
IEI	Idiopathic environmental intolerance
IEI-EMF	Idiopathic environmental intolerance attributed to electromagnetic fields
IgE	Immunoglobulin E
IgG	Immunoglobulin G
IQR	Interquartile ranges
LBT	Lactulose breath test
LC	Liquid chromatography
LDTs	Laboratory-developed tests
LHBT	Lactulose hydrogen breath test
MCS	Multiple chemical sensitivity
MS	Mass spectrometry
NASPGHAN	North American Society for Pediatric Gastroenterology, Hepatology and Nutrition
ONE	Optimal nutritional evaluation
PCBs	Polychlorinated biphenyls
PHQ-9	Patient Health Questionnaire-9
SIBO	Small intestinal bacterial overgrowth
VMA	Vanillylmandelic acid
WHO	World Health Organization

Scientific Background

Patients with idiopathic environmental intolerance (IEI) typically report sensitivity to multiple, chemically unrelated substances and become ill due to a wide range of nonspecific symptoms when exposed. Symptoms may include anxiety, shortness of breath, chest pain, and more. Psychiatric disorders may also be at the core of the IEI patient. The mean age of patients reporting IEI is between 30 and 40 years and individuals who are married are significantly more likely to be diagnosed with IEI than those who are

not. IEI also occurs in 40% of people with chronic fatigue syndrome and in 16% of people with fibromyalgia (Black & Temple, 2024; Black et al., 2024).

The symptoms of IEI are nonspecific, ambiguous and common in the general population. There is no characteristic set of symptoms and ultimately no major differences between patients self-reporting IEI and those that do not. Virtually any symptom can be considered a symptom of IEI (Black & Temple, 2024). Within the definition of multiple chemical sensitivity (MCS), identified symptoms included "asthmatic-like, skin irritation, dermatitis, migraine, dysuria, dyspepsia, symptoms of supposed sensitization to food, persistent arthromial pain, vertigo, vestibular impairment", with 80% of patients experiencing "asthenia, arthromial pain, dyspepsia, coriza, eructation, chest pain, insomnia" (Quarato et al., 2020). The classification of IEI as a distinct medical disorder is also in question, as a lack of reliable case reports, lack of consistent findings or laboratory results, and reliance on surveys or self-reporting all cloud the condition and understanding of this disorder (Black & Temple, 2024).

Recently, many articles have been published suggesting a relationship between electromagnetic fields and IEI. Electromagnetic fields may include radiofrequencies from telecommunication devices (Eltiti et al., 2018; Huang et al., 2018), Wi-Fi and base stations (ANSES, 2018). For an unknown reason, these individuals claim to react to the exposure of certain electromagnetic triggers that most people can tolerate without issues; these triggers are below established toxicological and hazardous thresholds. ANSES (2018) researched the relationship between electric field exposure and IEI symptoms and stated that "either the symptoms experienced by EHS [electromagnetic hypersensitivity] individuals are not caused by exposure to electromagnetic fields and there are no quantifiable biological and/or physiological abnormalities when they are exposed to electromagnetic fields (assumption one) or the absence of results is due to the methodological limitations of the provocation studies (subject selection, sample size, exposure type, etc.) (assumption two)". These findings were corroborated by Schmiedchen et al. (2019), who, in their systematic review of articles pertaining to EHS, stated, "limitations in design, conduct and analysis could therefore have given rise to either false positive for false negative results," and that the "nocebo effect or medical/mental disorders may explain the complaints in many individuals". Characteristic symptoms of EHS include sleep and circadian rhythm disorders, migraines and headaches, hypersensitivity, and other related syndromes and disorders such as fibromyalgia, tinnitus and MCS (ANSES, 2018).

Tests such as elimination diets, food challenges, and provocation-neutralization tests have been used to test for food or chemical sensitivities. Immunological tests or tests measuring the amount of various chemicals in body tissues have also been performed (Black & Temple, 2024). In fact, testing for a wide range of autoantibodies is generally discouraged, as "pretest probability is low, and false-positive results are far more likely than true-positive results; a weakly positive ANA [antinuclear antibodies] is present in about 20% of the population" (Black & Temple, 2024). However, these assessments are typically not rigorous enough to provide strong evidence; for example, these tests are often not performed blinded or with placebo controls. No unusual laboratory findings have been reliably linked to IEI (Black & Temple, 2024). Due to the vast number of causes, symptoms, responses, and general heterogeneity of this condition, it may be very difficult to provide a scientifically valid or useful test. Worse, testing may even exacerbate or increase the number of symptoms of a patient. Physicians should use caution in testing for reassurance of patients as negative findings may increase anxiety instead (Barsky & Borus, 1999; Black & Temple, 2024).

Proprietary Testing

Due to the number of symptoms that may be considered part of IEI, there are a corresponding number of tests performed. These tests are generally unnecessary as the condition itself is far too ambiguous to reliably test for and any test can be ordered under the guise of IEI. For example, assessment of factors such as elastase, stool culturing, or fat differentiation may all be done for the sake of IEI treatment. These tests may have legitimate medical purposes (for instance a stool culture may be useful for numerous conditions) but their use for IEI is essentially none, as IEI itself carries no reliable characteristics to test for. Other tests that evaluate a tangentially relevant analyte, such as micronutrient panels or a lactose intolerance breath test (BT), may be done for IEI's sake as well. Since virtually any symptom or sign can be called IEI, these tests are sometimes ordered for nonspecific or subjective symptoms such as fatigue or pain. However, these tests cannot provide any useful results because of the dubious nature of IEI itself.

Another commonly used test for IEI are panels that test multiple factors in one. For example, the Triad Bloodspot Profile offered by Genova Diagnostics measures organic acid levels, "the level of IgG4 reactions for 30 common foods," and "essential amino acid imbalances" (Genova, 2021c). Genova offers several similar panels, such as the Organix Comprehensive Profile (which tests 46 analytes for subjective symptoms such as depression, weight issues and chemical sensitivities) (Genova, 2022a), the NutrEval FMV [first morning void] (which tests 118 analytes for symptoms such as fatigue, weight issues, and sports fitness optimization) (Genova, 2021a) and the Allergix IgG4 Food Antibodies (which tests 90 foods for sensitivity). Genova Diagnostics also offers the GI Effects Profile (advanced stool tests for the management of gastrointestinal [GI] health), a full line of allergy testing and assessment tests (measuring IgG and IgE food antibodies, inhalants, molds and spices), the Ion Profile (which evaluates various types of organic, amino and fatty acids as well as nutrient and toxic elements), the Comprehensive Digestive Stool Analysis (CDSA) 2.0 Profile with Parasitology (evaluates the microbiome, digestion and absorption), and SIBO Profile tests (breath tests which measure methane gases and exhaled hydrogen) (Genova, 2022b).

The hydrogen breath test is used to assess lactose malabsorption. After ingesting a lactose solution, serial breath samples are taken to determine hydrogen levels. Lactose should be used in amounts ranging from 25 to 50 g for those aged 18 and up. There is no current consensus on the lactose dosage in children, with estimates ranging from 0.5 to 2 g/kg lactose suspended in water to a maximum of 25 to 50 g. Proper test performance needs the following: Cigarette smoking or physical activity that causes hyperventilation should be avoided for two hours before testing, since it can reduce test accuracy. Complex carbs (i.e. bread, pasta, and fiber) and dairy should be avoided for 12 hours before testing. Antibiotics should be avoided four weeks before testing. Colonic cleaning for endoscopic or surgical procedures should be avoided for at least two weeks before testing. The suggested test time is three to five hours; it may be completed sooner if a positive diagnosis of malabsorption is confirmed with the standard measuring interval for determining malabsorption being 30 minutes. However, longer intervals of up to 60 minutes might be appropriate (Hammer & Högenauer, 2024).

An evaluation of symptoms of IEI patients includes a history, physical examination, and laboratory tests (complete blood count, serum electrolytes and glucose, urine analysis) with further testing guided by reported symptoms. An occupational or environmental history is also useful as patients typically report problems from chemical exposure (Black & Temple, 2024). A questionnaire such as the "Environmental Exposure and Sensitivity Intolerance" (EESI) may be used for an initial screening (Rossi & Pitidis, 2018). A psychiatric history is also recommended as psychiatric disorders are often co-morbid with IEI. A screening questionnaire such as the Patient Health Questionnaire (PHQ-9) can be used to identify psychiatric conditions in an IEI patient (Black & Temple, 2024; Gilbody et al., 2007).

Micronutrients are the essential vitamins and minerals required by the body for proper functioning. Panels have been developed which evaluate intracellular levels of essential vitamins and minerals. These panels may also be used on IEI patients. This may help to identify nutritional deficiencies in otherwise healthy patients or in patients suffering from some type of disease. SpectraCell Laboratories have developed the Micronutrient Test Panel, which is able to measure 31 vitamins, minerals, metabolites, amino acids, fatty acids and antioxidants; this test also measures how these micronutrients affect cellular functioning in an individual (SpectaCell, 2024). SpectraCell Laboratories have also developed the SPECTROX™, claiming it measures total antioxidant function in an individual, reporting on the repair mechanisms and net ability of each individual's cells (SpectraCell, 2008). As noted above, Genova Diagnostics has developed the NutrEval FMV that measures 118 markers, including amino acids, fatty acids and organic acids (Genova, 2021a). ONE (Optimal Nutritional Evaluation) FMV, also by Genova Diagnostics, is a urine-based nutritional test which assesses "the functional need for antioxidants, B-vitamins, minerals, digestive support and amino acids" (Genova, 2021b). The company notes that the ONE FMV test may be used for patients with mood disorders, fatigue, digestive issues, weight problems, general health, dietary guidance and fitness. Another nutrient panel blood test, developed by Life Extension, measures vitamin B12, folate, vitamin D 25-hydroxy, vitamin A, vitamin C, selenium, zinc, CoQ10 (coenzyme Q10) and magnesium (LifeExtension, 2024). Finally, Vibrant America provides a test which measures approximately 40 intracellular and extracellular vitamins, minerals, fatty acids, amino acids and antioxidants (Vibrant, 2017).

Clinical Utility and Validity

Very little information suggests that the intracellular micronutrient analysis assists with positive health outcomes. Houston (2013) published an article on the role of vitamins, minerals and overall nutrition in the prevention and treatment of hypertension. This article reviewed hypertension-related clinical trials that include information on the "efficacy of nutrition, weight loss, exercise, and nutritional supplements, vitamins, minerals, and antioxidants" (Houston, 2013). Approximately 3338 individuals were treated with micronutrient testing over a five-year period, with 20% of these patients exhibiting abnormally high blood pressure. After six months, 62% of the hypertensive patients reached lower blood pressure goals. Hence, the author states that the diagnosis and treatment of various nutritional deficiencies can decrease the number of cardiac events as well as reduce blood pressure and improve vascular biology. However, data for the control group not treated with micronutrients was not provided for comparison.

Another technique that has been used to assess nutritional status is the measurement of the hepatic proteins prealbumin and albumin. However, it seems that a physical examination has evolved as the main technique to diagnose malnutrition in a clinical setting. "The current consensus is that laboratory markers are not reliable by themselves but could be used as a complement to a thorough physical examination" in a malnutrition diagnosis (Bharadwaj et al., 2016). The Academy of Nutrition and Dietetics (AND) also do not accept albumin and prealbumin as a diagnostic tool for malnutrition and state that "there is no laboratory test that is both sensitive to and specific for protein-calorie malnutrition" (AND, 2017).

Idiopathic environmental intolerance patients may also report bowel irritability. Small intestinal bacterial overgrowth (SIBO) occurs when excessive aerobic and anaerobic bacteria colonize the small intestine; these bacteria are not typically found in the colon and can cause chronic diarrhea and malabsorption (Pimentel, 2024). SIBO may be diagnosed by a breath test. However, a validated gold standard method for diagnosing SIBO has not been indicated (Rezaie et al., 2017). The SIBO breath test uses carbohydrates in a simple, non-invasive and widely available testing method. A carbohydrate substrate (such as lactulose or glucose) is administered to the patient, which leads to the production of an analyte

such as hydrogen or methane. “In individuals without SIBO, the administration of lactulose results in a single peak in breath hydrogen/methane within two to three hours due to the metabolism of lactulose by colonic flora. In patients with SIBO, administration of lactulose results in an early peak in breath hydrogen/methane levels due to metabolism by small bowel bacteria” (Pimentel, 2024). As noted above, Genova Diagnostics has developed the SIBO Profile test which is a two or three hour breath test that measures methane gases and exhaled hydrogen (Genova, 2022b). This test requires the patient to ingest a lactulose solution. “There are several limitations to breath tests as diagnostic test for SIBO. Rapid delivery of the test substrate to the colon (eg, in patients with short bowel syndrome) may lead to false-positive results, while gastrointestinal disorders where gastric emptying is delayed may cause a false-negative test. In general, the sensitivity and specificity of the breath test are low, and there is a poor correlation between the breath test and the small bowel aspiration and culture method” (Pimentel, 2024).

De Geyter et al. (2021) investigated individuals below the age of 18 years that had symptoms suggesting lactose intolerance. The study's goal is to assess the value of measuring both H2 and CH4 in the diagnosis of lactose intolerance. The study comprised 209 individuals under the age of 18, with the average age being 8.3 years, who had symptoms of lactose intolerance and were tested with lactose H2 and CH4 breath test. Over 90% experienced gastrointestinal issues, namely cramping or stomach discomfort, flatulence, bloating, and diarrhea. Ninety-six individuals (46%) in this group tested positive for H2 in their breath. A positive H2 breath test revealed lactose malabsorption in 46% of people under the age of 18. Significantly more CH4 producers were present in the group of H2 producers (5.7 vs. 14.8%; CHI square < 0.001), supporting the idea that high levels of H2 are required for CH4 creation. Six of the ten patients who excreted large quantities of CH4 (>20 ppm over baseline) also tested positive for the H2 test. Almost 15% of those with a positive H2 breath test (>20 ppm above baseline) also tested positive for CH4. The study found considerable CH4 generation in 5.7% of patients with a negative H2 test (De Geyter et al., 2021; Geyter et al., 2021).

Bratten et al. (2008) completed a study with 224 individuals with irritable bowel syndrome (IBS) and 40 controls. A lactulose breath test (LBT) was used to measure methane and hydrogen production to identify patients with IBS. Results showed that “The majority of patients with IBS and healthy subjects meet criteria for an "abnormal" LBT using previously published test criteria, and groups are not discriminated using this diagnostic method” (Bratten et al., 2008). The authors then questioned the utility of an LBT to diagnose IBS as the testing did not discriminate between IBS patients and healthy controls. A more recent study by Ghoshal et al. (2014) evaluated 80 patients with IBS for SIBO. Culture had previously diagnosed 15/80 patients with SIBO. Both lactulose and glucose hydrogen breath tests (LHBT and GHBT, respectively) were used to measure SIBO. The authors conclude that “the specificity of GHBT was 100%, but the sensitivity of this test and the diagnostic performances of LHBT and breath methane were all very poor” (Ghoshal et al., 2014).

Speck and Witthöft (2022) included 410 patients in a cross-sectional study design to investigate the relationship between IEI symptoms associated with chemicals and schizotypy spectrum. They found that “schizotypal traits were found to be significantly positively associated with [modern health worries], [chemical odor sensitivity]…, and showed significant positive associations with hallucination proneness. Magical thinking was found to exhibit a significant positive relationship with both [modern health worries] and [chemical odor sensitivity].” This demonstrates how the principles surrounding IEI may need to consider associated psychiatric differential diagnoses to properly evaluate symptoms and testing. Finding that patients have symptoms of chemical odor sensitivity and modern health worries can also conversely encourage further insight into the mental wellness of a patient.

Madigan et al. (2022) investigated the relationship between SIBO caused by Archaea and certain clinical symptoms. Archaea are anaerobic bacteria that produce methane specifically. Through a retrospective cross-sectional study, the researchers used glucose breath tests conducted for SIBO to correlate the bacteria to their phenotypic manifestations. From 1461 patients, they found that 33.1% were SIBO positive, with 38.8% producing only methane, 11.4% producing both methane and hydrogen, and 49.8% with hydrogen only producing organisms. Methane-producing SIBO patients had an increased odds of experiencing constipation and gassiness in comparison to SIBO(-) patients. On the other hand, hydrogen-producing SIBO patients had several "significant factors": "vitamin B12 deficiency (odds ratio, 1.44; CI, 1.01–2.06; P = .046), [Roux-en-Y Bypass] (odds ratio, 2.14; CI, 1.09–4.18; P = .027), cholecystectomy(odds ratio, 1.42; CI, 1.06–1.91; P = .020), , and diabetes (odds ratio, 1.59; CI, 1.13–2.24; P = .008)." However, when comparing methane-producing SIBO versus hydrogen-producing SIBO patients, "vitamin B12 deficiency was the only factor that reached significant (OR 0.57; CI, 0.34-0.97; P = 0.038), indicating that [methane-producing SIBO] patients were almost half as likely to report cobalamin deficiency." This study demonstrated the implications of varying gas producing organisms in SIBO and the clinical symptoms that can affect treatment and prognosis, solely by extrapolating data from breath tests (Madigan et al., 2022).

Rangan et al. (2022) conducted a review to investigate the clinical utility and drawbacks of SIBO breath testing. They identified that the "variability in oral-cecal transit time" was the biggest limitation in breath testing, and that it greatly contributed to common false-positive test results. This theoretically results from lactulose fermentation by normal colonic flora versus invasive microbial flora. In comparing the specificity and sensitivity for lactulose breath testing versus glucose breath testing, it was found that the former had a sensitivity of 42.0% and specificity of 70.6%, whereas the latter had a sensitivity of 54.5% and a specificity of 83.2%. However, those with a positive lactulose breath test result were more likely to respond to rifaximin therapy, thereby implying greater clinical utility. Despite the controversies in the substrates for testing, the researchers state that "notably, however, clinical symptoms have also been shown to be nonspecific for diagnosing SIBO, and thus breath testing remains a useful diagnostic tool in managing those patients with compatible symptoms and an absence of another diagnosis on endoscopy or imaging, particularly if there are other underlying conditions that could predispose to SIBO" (Rangan et al., 2022).

Bushyhead and Quigley (2022) corroborates the technical difficulties and clinical utility of SIBO breath testing discussed in the two studies mentioned above. In their review, they state that breath testing is less invasive and inexpensive relative to small bowel culture-based diagnoses. However, there is no solidified association between methanogenic overgrowth and gastrointestinal symptoms like constipation, as the "positive breath test for methane may be due to methane production by resident anaerobic colonic methanogens rather than small bowel flora." They also concur on the idea that "an important factor that may confound the interpretation of lactulose breath tests… is orocecal transit time…It is also possible that glucose malabsorption, which may be more prevalent than previously considered, could lead to a positive glucose breath test… Prior upper GI surgery could also contribute to accelerated orocecal transit of glucose; conversely, those with constipation and preformed gas can confound more test results." The variability and contamination limit the diagnostic utility of breath testing in the setting of SIBO (Bushyhead & Quigley, 2022).

Usai-Satta et al. (2021) conducted a literature review to study the usefulness of breath tests (BTs) in the nutritional management of abdominal pain, bloating, and diarrhea. The authors note that while BTs are inexpensive and can be simple to preform, there is a lack of standardization in the indications, preparation, performance, and interpretation of testing which results in "considerable heterogeneity between different centers and practitioners." For the management of lactose malabsorption and

intolerance, lactose BTs have "good sensitivity and optimal specificity," but are not accurate enough for a diagnosis. "An accurate diagnosis of lactose intolerance should require blind lactose challenge although this method is difficult to utilize in clinical practice." For the management of fructose malabsorption, there is "no gold standard available for fructose BT" and the authors found no significant validation studies to support the use of fructose BT. Similarly, for sorbitol malabsorption, there is no gold standard and no validation studies for the use of sorbitol BT. There are limited studies of BTs used for other carbohydrates including trehalose, maltitol, and sucrose, but there is "no sufficient evidence is available to recommend BTs related to these carbohydrates in clinical practice." The authors concluded that "blind sugar challenge remains the most valid technique to objectively demonstrate a clinical intolerance to carbohydrates" (Usai-Satta et al., 2021).

Guidelines and Recommendations

Due to the dubious nature of this condition, several prominent medical studies have regarded this condition with suspicion. In 1992, the American Medical Association (Anderson et al.) stated that multiple chemical sensitivity (now IEI) should not be recognized as a syndrome until accurate, reproducible, and well-controlled studies can be done (Coble et al., 1992). Other societies such as the American College of Physicians and the American Academy of Allergy and Immunology hold similar views (ACP, 1989; Anderson et al., 1986).

American Academy of Allergy, Asthma and Immunology (AAAAI)

In 2006, AAAAI referenced IEI in their position statement on the medical effects of mold stating that testing many nonvalidated immune based tests, as had been done to suggest an immunologic basis for IEI (MCS), is expensive, not useful or valid, and should be discouraged (Bush et al., 2006).

American College of Occupational and Environmental Medicine (ACOEM)

In 1999, the ACOEM published a position statement that stated there have been no consistent physical findings or laboratory abnormalities in IEI (then called MCS) patients and recommended that a generalized clinical approach, such as establishing a therapeutic alliance and avoiding unnecessary tests, would be useful in the management of other nonspecific medical syndromes (ACOEM, 1999).

French Agency for Food, Environmental and Occupational Health & Safety (ANSES) Appraisal-Collective Expertise Report

An ANSES expert committee published an opinion piece regarding the expert appraisal on EHS or IEI due to electromagnetic fields. This committee did not find any conclusive results regarding IEI and therefore does not recommend any specific testing methods for this ailment, other than the psychological testing of patients (ANSES, 2018).

Consensus Document (1999)

An international document, created by 89 clinicians and researchers with broad experience in the field, aimed to establish consensus criteria for MCS. The recognition criteria of MCS set forth by this expert panel are as follows:

- Chronic condition
- Reproducible symptoms with repeated chemical exposure
- Low exposure levels cause syndrome to occur

- Removal of offending agents cause symptoms to subside
- There are responses to chemically unrelated substances ("Multiple chemical sensitivity: a 1999 consensus," 1999)

The 1999 Consensus Document is the most widely used criteria for recognition of MCS (Martini et al., 2013).

North American Society for Pediatric Gastroenterology, Hepatology and Nutrition (NASPGHAN) and European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN)

The NASPGHAN and ESPGHAN have stated that "Clinicians should familiarize themselves with the limitations of nutritional biomarkers in the context of chronic liver disease" but do not give specific recommendations regarding nutritional laboratory testing (Mouzaki et al., 2019).

World Health Organization

The WHO published guidelines on the micronutrient intake in children with severe acute malnutrition. The guidelines recommend that the weight-for-height/weight-for-length status should be measured by clinicians to determine malnutrition. Micronutrient laboratory testing is not mentioned by the WHO (WHO, 2024).

The North American Expert Consensus Guidelines

A team of experts have published guidelines on breath tests including their use for a SIBO diagnosis. The authors have provided the following recommendations:

- "Current small bowel culture techniques are not satisfactory for the assessment of SIBO. [Quality of evidence: Low]
- If culture is considered for diagnosis of SIBO, based on the current evidence, we suggest the threshold of >10 3 c.f.u./ml for the definition of SIBO [Quality of evidence: Low]
- We suggest breath testing in the diagnosis of small intestinal bacterial overgrowth [Quality of evidence: Moderate]
- Until a true gold standard is established, we suggest breath testing in assessing the presence of antibiotic responsive microbial colonization of the gastrointestinal tract [Quality of evidence: Moderate]
- We suggest evaluating for excessive methane excretion on breath test in association with clinical constipation and slowing of gastrointestinal transit [Quality of evidence: Moderate]
- We suggest that breath testing should not be used for assessment of orocecal transit time [Quality of evidence: Moderate]
- We suggest breath testing for the diagnosis of carbohydrate maldigestion syndromes [Quality of evidence: Moderate]
- We suggest breath testing in the assessment of conditions with bloating [Quality of evidence: Low]
- We suggest that fructose and lactose breath test should be performed for at least 3 hours [Quality of evidence: Moderate]
- We suggest that the presence of bacterial overgrowth should be ruled out before performing lactose or fructose breath testing [Quality of evidence: Moderate]" (Rezaie et al., 2017).

It may be worth noting that the above recommendation of LHBT testing for SIBO was publicly criticized by Usai-Satta et al. (2018) due to high false positive rates and a low sensitivity. The authors state that "in

our opinion, LHBT should be neither recommended nor suggested to detect SIBO in the clinical practice. Despite a low sensitivity, Glucose BT [breath test] remains the most accurate BT for non-invasive diagnosis of SIBO" (Usai-Satta et al., 2018). In contrast, an article published in Gastroenterology by Baker et al. (2021) did a retroactive study, examining how these 2017 guidelines for glucose breath testing for SIBO compared to the older, modified Rome Consensus protocols. The authors found that the more recent North American Consensus protocol showed a higher percent of individuals with SIBO because of more prevalent positive methane excretion. Another article published by Pitcher et al. (2022) provide further support for the North American Consensus protocol for SIBO testing.

The Academy of Nutrition and Dietetics

The AND note that "serum proteins such as albumin and prealbumin are not included as defining characteristics of malnutrition because evidence analysis shows that serum levels of these proteins do not change in response to changes in nutrient intake. Hepatic proteins are not indicators of nutritional status, but are rather indicators of morbidity and mortality, and recovery from acute and chronic disease" (AND, 2017).

American College of Gastroenterology (ACG)

The ACG published an update on SIBO (Small Intestinal Bacterial Overgrowth). This guideline addresses diagnostic testing and treatment options for SIBO. Their recommendations include:

- "We suggest the use of breath testing (glucose hydrogen or lactulose hydrogen) for the diagnosis of SIBO in patients with IBS (conditional (weak) recommendation, very low level of evidence)."
- "We suggest using glucose hydrogen or lactulose hydrogen breath testing for the diagnosis of SIBO in symptomatic patients with suspected motility disorders (conditional (weak) recommendation, very low level of evidence)."
- "We suggest testing for SIBO using glucose hydrogen or lactulose hydrogen breath testing in symptomatic patients (abdominal pain, gas, bloating, and/or diarrhea) with previous luminal abdominal surgery (conditional (weak) recommendation, very low level of evidence)."
- "We suggest testing for methane using glucose or lactulose breath tests to diagnose the overgrowth of methane-producing organisms (IMO) in symptomatic patients with constipation (conditional (weak) recommendation, very low level of evidence)."

The ACG also notes that although "Small bowel aspirate and culture is often considered the gold standard for the diagnosis of SIBO," there have been some preliminary studies focusing on use of nucleic acid testing to diagnose SIBO. However, the ACG remarks that "Large-scale studies are currently underway to evaluate this further" (Pimentel et al., 2020).

Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

No specific U.S. Food and Drug Administration (FDA) approval or clearance of a test for idiopathic environmental intolerance was found. Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
82108	Aluminum
82127	Amino acids; single, qualitative, each specimen
82136	Amino acids, 2 to 5 amino acids, quantitative, each specimen
82139	Amino acids, 6 or more amino acids, quantitative, each specimen
82300	Cadmium
82379	Carnitine (total and free), quantitative, each specimen
82380	Carotene
82441	Chlorinated hydrocarbons, screen
82495	Chromium
82507	Citrate
82525	Copper
82542	Column chromatography, includes mass spectrometry, if performed (eg, HPLC, LC, LC/MS, LC/MS-MS, GC, GC/MS-MS, GC/MS, HPLC/MS), non-drug analyte(s) not elsewhere specified, qualitative or quantitative, each specimen
82653	Elastase, pancreatic (EL-1), fecal; quantitative
82656	Elastase, pancreatic (EL-1), fecal, qualitative or semi-quantitative
82705	Fat or lipids, feces; qualitative
82710	Fat or lipids, feces; quantitative
82715	Fat differential, feces, quantitative
82726	Very long chain fatty acids
82978	Glutathione
83015	Heavy metal (eg, arsenic, barium, beryllium, bismuth, antimony, mercury); qualitative, any number of analytes
83018	Heavy metal (eg, arsenic, barium, beryllium, bismuth, antimony, mercury); quantitative, each, not elsewhere specified
83150	Homovanillic acid (HVA)
83497	Hydroxyindolacetic acid, 5-(HIAA)
83655	Lead
83735	Magnesium
83785	Manganese
83885	Nickel
83918	Organic acids; total, quantitative, each specimen
83919	Organic acids; qualitative, each specimen
83921	Organic acid, single, quantitative
84134	Prealbumin
84255	Selenium
84446	Tocopherol alpha (Vitamin E)

CPT	Code Description
84585	Vanillylmandelic acid (VMA), urine
84590	Vitamin A
84600	Volatiles (eg, acetic anhydride, diethylether)
84630	Zinc
86001	Allergen specific IgG quantitative or semiquantitative, each allergen
86353	Lymphocyte transformation, mitogen (phytomitogen) or antigen induced blastogenesis
89125	Fat stain, feces, urine, or respiratory secretions
91065	Breath hydrogen or methane test (eg, for detection of lactase deficiency, fructose intolerance, bacterial overgrowth, or oro-cecal gastrointestinal transit)
S3708	Gastrointestinal fat absorption study

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

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Revision History

Revision Date	Summary of Changes
12/04/2024	Reviewed and Updated: Updated the background, guidelines and recommendations, and evidence-based scientific references. Literature review did not necessitate any modifications to coverage criteria.

Diagnosis of Vaginitis

Policy Number: AHS – M2057 – Diagnosis of Vaginitis	Prior Policy Name and Number, as applicable: AHS – M2057 – Diagnosis of Vaginitis including Multi-target PCR Testing
Initial Presentation Date: 09/18/2015 Revision Date: 03/06/2024	

Policy Description

Vaginitis is defined as inflammation of the vagina with symptoms of discharge, itching, and discomfort often due to a disruption of the vaginal microflora. The most common infections are bacterial vaginosis, *Candida* vulvovaginitis, and trichomoniasis (Sobel, 1999). Other causes include vaginal atrophy in postmenopausal women, cervicitis, foreign body, irritants, and allergens (Sobel, 2023b).

Bacterial vaginosis (BV) is characterized by a shift in microbial species from the normally dominant hydrogen-peroxide producing *Lactobacillus* species to *Gardnerella vaginalis* and anaerobic commensals (Eschenbach et al., 1989; Hill, 1993; Lamont et al., 2011; Ling et al., 2010; Sobel, 2023a).

Vulvovaginal candidiasis (VVC) is usually caused by *Candida albicans* but can occasionally be caused by other *Candida* species (CDC, 2021c). It is the second most common cause of vaginitis symptoms (after BV) and accounts for approximately one-third of vaginitis cases (Sobel & Mitchell, 2023a; Workowski & Bolan, 2015).

Trichomoniasis is caused by the flagellated protozoan *Trichomonas vaginalis*, which principally infects the squamous epithelium in the urogenital tract: vagina, urethra, and paraurethral glands (Kissinger, 2015; Sobel & Mitchell, 2023b).

Related Policies

Policy Number	Policy Title
AHS-G2002	Cervical Cancer Screening
AHS-G2149	Pathogen Panel Testing
AHS-G2157	Diagnostic Testing of Common Sexually Transmitted Infections
AHS-M2097	Identification of Microorganisms Using Nucleic Acid Probes

Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) For individuals with symptoms of vaginitis, testing of pH, testing for the presence of amines, saline wet mount, hydrogen peroxide (KOH) wet mount, and microscopic examination of vaginal fluids **MEETS COVERAGE CRITERIA**.

2) For individuals with symptoms of vaginitis, direct probe DNA-based identification of *Gardnerella*, *Trichomonas*, and *Candida* (e.g., BD Affirm™ VPIII) **MEETS COVERAGE CRITERIA**.

3) For individuals with clinical signs and symptoms of vaginitis but with negative findings on wet-mount preparations and a normal pH test, vaginal cultures for *Candida* species for the diagnosis of vulvovaginal candidiasis **MEET COVERAGE CRITERIA**.

4) For individuals with symptoms of vaginitis, measurement of sialidase activity in vaginal fluid for the diagnosis of bacterial vaginosis **MEETS COVERAGE CRITERIA**.

5) For individuals with symptoms of vaginitis, nucleic acid amplification testing (NAAT) or polymerase chain reaction (PCR)-based identification of *Trichomonas vaginalis* **MEETS COVERAGE CRITERIA**.

6) For individuals with risk factors for trichomoniasis (new or multiple partners; history of sexually transmitted infections (STIs), especially HIV; exchange of sex for payment; incarceration; injection drug use), screening for *Trichomonas* **MEETS COVERAGE CRITERIA**.

7) For individuals with complicated vulvovaginal candidiasis (VVC), polymerase chain reaction (PCR) based identification of *Candida* to confirm clinical diagnosis and identify non-albicans *Candida* **MEETS COVERAGE CRITERIA**.

8) For individuals with symptoms of bacterial vaginosis (BV), NAAT specific to the diagnosis of BV (e.g., Aptima® BV; OneSwab® BV Panel PCR with Lactobacillus Profiling by qPCR; SureSwab® Advanced BV, TMA) and single or multitarget PCR testing for the diagnosis of BV **MEETS COVERAGE CRITERIA**.

9) NAAT panel testing designed to detect more than one type of vaginitis (VVC, BV, and/or trichomoniasis; e.g., BD MAX™ Vaginal Panel, NuSwab® VG, Xpert® Xpress MVP) **MEETS COVERAGE CRITERIA**.

10) For asymptomatic individuals, including asymptomatic pregnant individuals at an average or high risk for premature labor, screening for trichomoniasis and bacterial vaginosis **DOES NOT MEET COVERAGE CRITERIA**.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

11) For individuals with symptoms of vaginitis, rapid identification of *Trichomonas* by enzyme immunoassay **DOES NOT MEET COVERAGE CRITERIA**.

12) Testing for microorganisms involved in vaginal flora imbalance and/or infertility using molecular-based panel testing **DOES NOT MEET COVERAGE CRITERIA**.

13) All other tests for vaginitis not addressed above **DO NOT MEET COVERAGE CRITERIA**.

Table of Terminology

Term	Definition
AAFP	American Academy of Family Physicians
ACOG	American College of Obstetrics and Gynaecology
ASM	American Society for Microbiology
AV	Aerobic vaginitis
BV	Bacterial vaginosis
BVAB	BV associated bacteria
CDC	Centers for Disease Control and Prevention
CLIA	Clinical Laboratory Improvement Amendments
CMS	Centers for Medicare and Medicaid
DNA	Deoxyribose nucleic acid
DOS	Date of service
HIV	Human Immunodeficiency Virus
IDSA	Infectious Diseases Society of America
LDTs	Laboratory developed tests
MDL	Medical Diagnostic Laboratories
NAAT	Nucleic acid amplification testing
NPV	Negative predictive value
OADS	Office of the Associate Director for Science
PCR	Polymerase chain reaction
PMNs	Polymorphonuclear cells
PPV	Positive predictive value
RTPCR	Real-time polymerase chain reaction
SOGC	Society Of Obstetricians and Gynaecologists of Canada
STDs	Sexually transmitted diseases
TMA	Transcription-mediated amplification
TV	Trichomonas vaginalis
USPSTF	U.S. Preventive Services Task Force
VVC	Vulvovaginal candidiasis

Scientific Background

Vaginitis is characterized by several symptoms including odor, itching, abnormal vaginal discharge, burning and irritation; this inflammatory ailment is considered the most common gynecologic diagnosis in primary care as most women experience vaginitis at least once in their lives (Paladine & Desai, 2018). A diagnosis of vaginitis can be given based on a combination of symptoms, physical examination, and office or laboratory-based testing methods.

The squamous epithelium of the vagina in premenopausal women is rich in glycogen, a substrate for lactobacilli, which create an acidic vaginal environment (pH 4.0 to 4.5). This acidity helps maintain the normal vaginal flora and inhibits growth of pathogenic organisms. Disruption of the normal ecosystem by menstrual cycle, sexual activity, contraceptive, pregnancy, foreign bodies, estrogen level, sexually transmitted diseases, and use of hygienic products or antibiotics can lead to development of vaginitis. Bacterial vaginosis (BV), vulvovaginal candidiasis (VVC), and trichomoniasis are the three most common infections responsible for vaginitis. Other causes include: vaginal atrophy in postmenopausal women, cervicitis, foreign body, irritants and allergens (Sobel, 2023b).

Bacterial vaginosis is caused by an imbalance of naturally occurring vaginal bacteria, characterized by both a change in the most common type of bacteria present, along with an increase in the total number of bacteria present. Normal vaginal microbiota is dominated by the species *Lactobacilli*, which are known to produce hydrogen peroxide and lactic acid, which help to keep the acidic vaginal environment below pH 4.5 (Jones, 2019; Kairys & Garg, 2023). Though the origin of vaginal bacterial infections is still unclear, it is believed that most of such infections are the result of another bacteria, *Gardnerella vaginalis,* creating a biofilm which allows opportunistic bacteria to grow within the vagina, causing a decrease in the *Lactobacilli* and subsequent disruption of the pH of the system. An entire host of etiologic organisms have been identified as possible instigators and exacerbators, including *Atopobium vaginae*, *Megasphaera* phylotype 1 and 2, *Leptotrichia aminionii*, *Mobiluncus spp*, *Prevotella spp*, *Mycoplasma hominis*, *Bacteroides spp*, *Sneathia*, and BV-associated bacteria (BVAB) 1, 2, and 3, though as aforementioned the causative mechanism and the interaction between these species are still uncertain (Jones, 2019).

Laboratory documentation of the etiology of vaginitis is important before initiating therapy, given the nonspecific nature and considerable overlap of the symptoms (Anderson et al., 2004; Ellis et al., 2001; Landers et al., 2004). Diagnostic testing enables targeted treatment, increases therapeutic compliance, and increases the likelihood of partner notification (Sobel, 2023b; Workowski & Bolan, 2015).

Measurement of vaginal pH is the primary initial finding that drives the diagnostic. The pH of the normal vaginal secretions in premenopausal women with relatively high estrogen levels is 4.0 to 4.5. The pH of normal vaginal secretions in premenarchal and postmenopausal women in whom estrogen levels are low is ≥4.7. An elevated pH in a premenopausal woman suggests infections, such as BV (pH>4.5) or trichomoniasis (pH 5 to 6) and helps to exclude *Candida* vulvovaginitis (pH 4 to 4.5). Vaginal pH may also be altered by lubricating gels, semen, douches, intravaginal medications and in pregnant women, leakage of amniotic fluid (Anderson et al., 2004; Sobel, 2023b).

There are several challenging aspects to the diagnosis of the etiology of vaginitis based on clinical symptoms. Vaginitis is a global term for nonspecific syndrome and must be narrowed down to the distinct causative factors. Traditional methods have included microscopy, pH testing, amine 'whiff' test, and the Amsel criteria, depending on the suspected etiology. However, physicians may find in-office microscopy to be unavailable, time-consuming, and/or inconclusive in achieving a diagnosis – some

estimates hold that misdiagnosis of vulvovaginitis approaches 50% (Brown & Drexler, 2020). As another confounding factor, coinfections are common in vaginitis, adding difficulty in diagnosis of the three most common organisms if there is mixed vaginitis or coinfection (Sobel, 2023b).

Even though studies have shown that PCR methods have a higher specificity and sensitivity than culture and shorter turn-around time in identifying *Candida* (Diba et al., 2012; Mahmoudi Rad et al., 2012; Tabrizi et al., 2006; Weissenbacher et al., 2009), their use may be adding to clinical non-specificity. Tabrizi et al. (2006) reported that PCR "detected four additional *Candida albicans*, three *Candida parapsilosis* and one *Candida tropicalis* when compared with culture. All but one case additionally detected by PCR were found in patients with no VVC symptoms (Tabrizi et al., 2006)." These data support the earlier findings by Giraldo et al. (2000) where, unlike culture testing, "*Candida* was identified by PCR in a similar proportion of patients with previous recurrent vulvovaginal candidiasis (30%) and in controls (28.8%)." Taken together, these studies indicate that, even though PCR is more sensitive than culture, it may be identifying cases of *Candida* in asymptomatic women that are clinically irrelevant.

Overall, microscopy has lower sensitivities and negative predictive values for BV, candidiasis, and trichomoniasis, and yeast when compared to NAAT and culture, respectively (Sobel, 2023b). The use of established molecular diagnostic tests as an alternative to traditional methods is an opportunity to improve the diagnosis and management of vaginitis; NAAT tests have already improved detection of trichomoniasis (Sobel, 2023b).

Proprietary Tests

DNA hybridization probe tests

As previously stated, microscopy, rather than bacterial culture, is the standard of care for diagnosing BV, and commercially available tests are available in the absence of microscopy but are not widely used. A study of 176 women using the Affirm VP III test (a DNA hybridization probe test that identifies high concentrations of *G. vaginalis*) reported comparable results to wet mount examination with no false positives and only three false negatives for *T. vaginalis,* and three false positives and four false negatives for *G. vaginalis* (Briselden & Hillier, 1994). This test "takes less than one hour to perform and is the best option when findings on physical examination suggest BV… but microscopy cannot be performed to look for clue cells (Sobel, 2023a)."

Trichomoniasis

The OSOM *Trichomonas vaginalis* (TV) Rapid Test by Sekisui Diagnostics is "an antigen-detection test that uses immunochromatographic capillary flow dipstick technology that can be performed at the POC [point of care]"(CDC, 2022). The diagnostic accuracy of the OSOM TV Rapid assay was tested against the common laboratory-based Anyplex II STI-7 Detection in a South African cross-sectional study; all irregular results were further tested with the Fast Track Diagnostics (FTD) STD9 assay (Garrett et al., 2019). Vaginal swabs from 247 women were tested for this study. "The sensitivity and specificity of OSOM TV were 75.0% (45.0-100) and 100% (100-100)", respectively, showing a very high specificity and lower sensitivity (Garrett et al., 2019).

Bacterial Vaginosis tests

AMPLISwab™

The AMPLISwab™ by MedLabs is a comprehensive test created to assess the different organisms responsible for a variety of female genital tract infections, including causative pathogens for cervicitis, nongonococcal urethritis, pelvic inflammatory disease and infertility, sexually transmitted infections, and vaginitis (e.g., bacterial vaginosis, candidiasis and trichomoniasis). The test requires one swab to test for 23 total organisms, broken down into four categories (seven yeast, 12 bacteria and one reference bacteria, one parasite, and two types of herpes viruses), employing testing methodologies such as automated DNA/RNA extraction, transcription-mediated amplification (Schwebke et al.), and real-time polymerase chain reaction (RT-PCR) for the quantification of select organisms implicated in bacterial vaginosis (MedLabs, 2015).

Aptima® BV

The Aptima® Assay by Hologic is a NAAT that identifies BV. "NAAT detects 3x more mixed infections cases than clinical diagnosis with wet mount and Amsel's criteria" (Hologic, 2024b). The Aptima BV Assay is a NAAT that utilizes real time transcription-mediated amplification (Schwebke et al., 2020) for the detection and quantification of ribosomal RNA from BV-associated bacteria: *Lactobacillus* (*L. gasseri*, *L. crispatus*, and *L. jensenii*), *Gardnerella vaginalis*, and *Atopobium vaginae*. "The assay reports a qualitative result for BV and does not report results for individual organisms. The assay is intended to aid in the diagnosis of BV on the automated Panther system using clinician-collected and patient-collected vaginal swab specimens from females with a clinical presentation consistent with vaginitis and/or vaginosis" (FDA, 2019a).

OneSwab®

OneSwab® by Medical Diagnostic Laboratories (MDL) uses real-time PCR and qPCR to output a graphical representation of the relative concentrations of the microbial flora. The Bacterial Vaginosis (with *Lactobacillus* profiling) qPCR test results are then reported in a text based and graphical format. The graphic format includes a representation of the results of all the quantitative tests included in the panel. The relative ratios of DNA species in the give sample in proportion to one another reflect the relative concentrations of different bacteria in vaginal specimens. According to the website, the panel includes assays to detect *Gardnerella vaginalis* and *Atopobium vaginae,* which are established BV organisms. NAAT is 95% sensitive and 99% specific for these organisms. In addition, two new assays to detect *Megasphaera* species and *Bacterial Vaginosis-Associated Bacterium 2* (BVAB2) are included in the Bacterial Vaginosis (with *Lactobacillus* profiling) panel. According to MDL, using NAAT to detect either of these two organisms is up to 99% sensitive and 94% specific for the diagnosis of BV when compared to Amsel Criteria and Nugent Score (MDLabs, 2022). Of note, the sensitivity and specificity just described are for the use of NAAT in detecting these microorganisms, as reported by Fredricks et al. (2007), and are not necessarily the sensitivity and specificity of the MDL *OneSwab®* for BV.

SureSwab® Advanced Bacterial Vaginosis (BV), TMA

The SureSwab® (Quest Diagnostics, Inc.) Advanced Bacterial Vaginosis (BV), TMA uses real time TMA to screen for microorganisms involved in BV vaginal flora imbalances, including *Lactobacillus* species, *Atopobium vaginae*, and *Gardnerella vaginalis* from a single vaginal swab. It reports a qualitative result for BV and does not report results for individual organisms. The swab can be collected either by a physician or the patient (Quest, 2022a).

OSOM® BVBlue®

The *OSOM® BVBlue®* chromogenic diagnostic point-of-care test is a CLIA-waived test with a reported 10 minute read time. The test detects "elevated vaginal fluid sialidase activity, an enzyme produced by bacterial pathogens associated with bacterial vaginosis including *Gardnerella, Bacteroides, Prevotella,* and *Mobiluncus*. 92.8% sensitive, 98% specific versus Gram Stain with a 1-minute hands-on-time, and instant color change provides clear easy-to-read results" (Diagnostics, 2023).

Combination panel tests for Vaginitis/Vaginosis

Aptima® CV/TV

Aptima® CV/TV assays are NAAT tests that identify "vulvovaginal candidiasis (Candida vaginitis or CV) and Trichomoniasis (Trichomonas vaginalis or TV) in symptomatic women from one vaginal sample. NAAT detects 3x more mixed infections cases than clinical diagnosis with wet mount and Amsel's criteria. These tests detect and qualitatively report results for the following organisms: *Candida* species group (*C. albicans, C. tropicalis, C. parapsilosis, C. dubliniensis*), *Candida glabrata*, *Trichomonas vaginalis*" (Hologic, 2024b).

SureSwab®

SureSwab® Advanced Vaginitis, TMA is a test for "vaginitis, including bacterial vaginosis, vulvovaginal candidiasis (*Candidiasis* species), and trichomoniasis (*Trichomonas vaginalis*) (Quest, 2022c). In an even more expansive combination test package, Quest offers a "SureSwab® Advanced Vaginitis Plus, TMA" assay which, in addition to detecting organisms associated with BV, trichomoniasis, and candidiasis, also detects *Chlamydia trachomatis* and *Neisseria gonorrhoeae* (Quest, 2022b).

BD MAX™ Vaginal Panel

The BD MAX™ Vaginal Panel is "an automated qualitative *in vitro* diagnostic test for the direct detection of DNA targets from bacteria associated with BV (qualitative results reported based on detection and quantitation of targeted organism markers), *Candida* species associated with vulvovaginal candidiasis, and *Trichomonas vaginalis* from vaginal swabs in patients who are symptomatic for vaginitis/vaginosis. The test utilizes real-time PCR for the amplification of specific DNA targets and utilizes fluorogenic target-specific hybridization probes to detect and differentiate DNA" (FDA, 2016).

Analytical Validity

Microscopic examination of normal vaginal discharge reveals a predominance of squamous epithelial cells, rare polymorphonuclear leukocytes (PMNs), and *Lactobacillus* species. The primary goal of the examination is to look for candidal buds or hyphae, motile trichomonads, epithelial cells studded with adherent coccobacilli (clue cells), and increased numbers of PMNs (Sobel, 2023b). The microscopic evaluation of BV is usually based on Amsel criteria (Amsel et al., 1983). Amsel criteria state that the presence of at least three out of the following four criteria are indicative of a BV diagnosis: increased homogeneous thin vaginal discharge, pH secretion > 4.5, amine odor when potassium hydroxide 10% solution is added to a vaginal secretion sample, and the presence of clue cells in wet preparations (Amsel et al., 1983). If clinical criteria are used to define infection, then reported sensitivity may range from 62 to 100 percent (Spiegel, 1991). Using Gram's stain as the standard for diagnosing BV, the sensitivity of Amsel criteria for diagnosis of BV is over 90 percent and specificity is 77 percent (Landers et al., 2004). The Nugent score is also available as a Gram staining scoring system to diagnose BV based on vaginal swab samples (Amegashie et al., 2017). Because BV represents complex changes in the vaginal flora, vaginal culture has **no** role in diagnosis. If microscopy is not available, commercial diagnostic

testing methods (e.g., rapid antigen and nucleic acid amplification tests) are used for confirming the clinical suspicion of BV. Polymerase chain reaction (PCR)-based assays to quantify BV-associated bacteria (Cartwright et al., 2012; Menard et al., 2008) have good sensitivity and specificity compared with standard clinical tests (Dumonceaux et al., 2009; Menard et al., 2010). However, they are expensive and of limited utility (Sobel, 2023a).

Trichomoniasis can be diagnosed by the presence of motile trichomonads on wet mount, but it is identified in only 60 to 70 percent of culture-confirmed cases. Culture on Diamond's medium was considered the gold standard method for diagnosing a *T. vaginalis* infection (Workowski & Bolan, 2015); however, nucleic acid amplification tests (Baron et al., 2013) have become the accepted gold standard for the diagnosis of *T. vaginalis*. One study found the sensitivities for *T. vaginalis* using wet mount, culture, rapid antigen testing, and transcription-mediated amplification testing were 65, 96, 90, and 98 percent, respectively (Huppert et al., 2007). Coexistence of *T. vaginalis* and BV pathogens is common, with coinfection rates of 60 to 80 percent (Sobel & Mitchell, 2023b; Sobel et al., 2013).

Microscopy is negative in up to 50 percent of patients with culture-confirmed VVC (Sobel, 1985). Since there are no reliable point of care tests for *Candida* available in the United States (Abbott, 1995; Chatwani et al., 2007; Dan et al., 2010; Hopwood et al., 1985; Marot-Leblond et al., 2009; Matsui et al., 2009), culture must be obtained. PCR methods have high sensitivity and specificity and a shorter turn-around time than culture (Diba et al., 2012; Mahmoudi Rad et al., 2012; Tabrizi et al., 2006; Weissenbacher et al., 2009), but they are costly and offer no proven benefit over culture in symptomatic women (Sobel & Mitchell, 2023a).

Lynch et al. (2019) collected vaginal swabs from 93 women in a cross-sectional study; results from microscopy were compared to two molecular approaches (a qPCR assay with a BV interpretive algorithm and a microbiome profiling test of the 16S rRNA gene produced by Illumina) (Lynch et al., 2019). Results show that "Microscopy plus BV Nugent score had 76% overall agreement with the qPCR plus BV interpretive algorithm method"; further, "Microscopic identification of *Candida* versus that by qPCR had 94% agreement (9 positive, 78 negative) (Lynch et al., 2019)." The qPCR assays gave additional information regarding the types of bacteria present, and the 16S microbiome analysis identified differentiating patterns between BV, aerobic vaginitis (AV), and *Lactobacillus* type infections.

Cartwright et al. (2018) have published data regarding the clinical validity of a PCR-based assay for the detection of BV. This multicenter study included 1579 patients and compared PCR results to samples realized by both the Nugent gram stain and a clinical evaluation using Amsel criteria. Next-generation sequencing was used to confirm differing results. After the resolution of discordant test results using next-generation sequencing, the BV-PCR assay reported a sensitivity of 98.7%, a specificity of 95.9%, a positive predictive value of 92.9% and a negative predictive value of 96.9% (Cartwright et al., 2018). These results show that this PCR-based assay can diagnose BV in symptomatic women efficiently.

Gaydos et al. (2017) conducted a cross-sectional, multi-site study into the clinical validation of this system (n=1740 symptomatic women) reported a sensitivity and specificity of 90.9% and 94.1%, respectively for the *Candida* group and 90.5% sensitivity and 85.8% specificity for BV. For *C. glabrata* specifically, the assay had only 75.9% sensitivity but 99.7% specificity. For trichomoniasis, the sensitivity and specificity were 93.1% and 99.3%, respectively (Gaydos et al., 2017). These researchers also compared the results of this test to clinician assessment. Again, to qualify for the study, the women must have at least one symptom of BV. Using Amsel's criteria, the investigational test sensitivity was 92.7% as compared to the 75.6% sensitivity of the clinician assessment. The authors conclude, "The investigational test showed significantly higher sensitivity for detecting vaginitis, involving more than one cause, than

did clinician diagnosis. Taken together, these results suggest that a molecular investigational test can facilitate accurate detection of vaginitis (Schwebke et al., 2018)." It should be noted, however, that these studies only included symptomatic women, and, therefore, the possible clinical non-specificity (i.e., instances where an asymptomatic woman would test positive) is not addressed. Sherrard (2019) compared BV, candidiasis, and trichomoniasis diagnostic results from the BD MAX Vaginal Panel to a current test used in a UK specialist sexual health service center. The authors reported that the BD MAX Vaginal Panel had a sensitivity of 86.4% and specificity of 86.0% for *Candida* species, and a sensitivity of 94.4% and specificity of 79% for BV; the specificity for BV was lower in this study than what has been previously reported (Sherrard, 2019).

Sumeksri et al. (2005) conducted a study correlated to the *OSOM® BVBlue®* test. 173 pregnant women reported a sensitivity and specificity of 94% and 96% respectively, as compared to Gram stain score. These results were comparable to the previously reported values of 91.7% sensitivity and 97.8% specificity in an earlier, smaller study of non-menstruating women (n=57) (Myziuk et al., 2003). A larger study (n=288 women) reported a sensitivity of 88% and specificity of 91% as compared to the Amsel criteria. The authors of this report concluded that women who "are not in settings where the conventional diagnostic methods are either practical or possible… would greatly benefit from access to rapid and reliable point-of-care tests to improve the diagnosis and management of BV (Bradshaw et al., 2005)."

Clinical Utility and Validity

Anand et al. (2020) investigated the accuracy of Papanicolaou smear to diagnose bacterial vaginosis infection in women with women with clinically evident genital infection using the Nugent score on Gram-stained smear as the gold standard. In a prospective blinded cross-sectional study of 254 nonpregnant women between the ages of 30 and 50 conducted between August 2016 and August 2018, the researchers found that using the Nugent score for diagnosing BV as the gold standard, the Pap smears showed sensitivity and specificity of 70.9% (CI: 61.5% - 79.2%) and 56.8% (CI: 48.2% - 65.2%), respectively. Moreover, they found that the positive percent value was 56.5% (CI: 47.8% - 64.9%), while the negative percent value was 71.2% (CI: 61.8% - 79.4%). These results indicated to the authors that though Pap smears are generally reserved for cervical cancer, the "Pap smear may serve as a means of diagnosing BV [bacterial vaginosis] infection in resource-constrained countries like India" (Anand et al., 2020).

Hilbert et al. (2016) performed a prospective longitudinal study on the use of molecular assays for the accurate detection and diagnosis of bacterial vaginosis using MDL *OneSwab®*. The authors quantified nine organisms associated with vaginal health or disease (*Gardnerella vaginalis, Atopobium vaginae, BV-associated bacteria 2 (BVAB2,* an uncultured member of the order *Clostridiales), Megasphaera phylotype 1 or 2, Lactobacillus iners, Lactobacillus crispatus, Lactobacillus gasseri,* and *Lactobacillus jensenii*) in a total of 149 women were enrolled in the study. DNA was extracted from clinical specimens using mechanical disruption and the QIAamp mini-kit from Qiagen; qPCR assay was used to quantify BV microbes and *Lactobacillus* species. Though the authors evaluated a broad variety of organisms with the potential to be diagnostic markers, results from the study indicated a sensitivity of 92% and specificity of 95% for three that were predictive of diagnosis of BV: *G. vaginalis*, *A. vaginae*, and *Megasphaera phylotypes 1 and 2;* outcomes were 94% PPV, and 94% NPV for BV. The authors summarized their findings by describing the molecular assay as a highly specific laboratory test to identify bacterial vaginosis (Hilbert et al., 2016).

The Aptima BV and Aptima Candida/Trichomonas vaginitis (CV/TV) NAAT molecular tests detect and qualitatively report results using a proprietary algorithmic analysis. Pathogens addressed by the test include: *Candida* species group (*C. albicans, C. tropicalis, C. parapsilosis, C. dubliniensis*), *Candida glabrata*, *Lactobacillus*, *Gardnerella vaginalis*, *Atopobium vaginae*, and *Trichomonas vaginalis* (Hologic, 2024a). Hologic announced the FDA approval of the Aptima BV and Aptima CV/TV vaginitis tests in 2019 (Hologic, 2019). Schwebke et al. (2020) performed a multicenter, prospective clinical study to validate the performance of the Aptima BV and Aptima CV/TV test for bacterial vaginosis, vulvovaginal candidiasis, and trichomonas vaginitis. A total of 1,519 subjects were enrolled in the study. The authors reported sensitivity and specificity for the investigational tests when it came to provider-collected samples at 95.0% and 89.6% for BV. When it came to *Candida* species, sensitivity and specificity was 91.7% and 94.9% respectively; *C. glabrata* sensitivity and specificity was 84.7% and 99.1%; 96.5% and 94.1% for *T. vaginalis*. Patient-collected samples showed similar ranges of sensitivity and specificity. In conclusion, the authors wrote, "In a secondary analysis, clinicians' diagnoses, in-clinic assessments, and investigational-assay results were compared to gold standard reference methods. Overall, the investigational assays had higher sensitivity and specificity than clinicians' diagnoses and in-clinic assessments, indicating that the investigational assays were more predictive of infection than traditional diagnostic methods" (Schwebke et al., 2020).

There has been increasing literature and reviews regarding both NAAT and DNA hybridization probe proprietary-based diagnostic performance in the identification of bacterial vaginosis. A study by Richter et al. (2019) compared the performance of three molecular diagnostic assays. The assays included in the study were BD Affirm, Hologic ASR BV Assay, and the Aptima IVD BV Assay. A total of 111 women were enrolled in the study. Women had been given an Affirm test by their provider after describing symptoms that indicated a form of vaginitis. After the collection of additional specimens, samples were run on the different assays. As predicted by clinicians, BV was the most common outcome of diagnosis for 45 of the patients (71%). The sensitivity and specificity for the Hologic ASR assay (diagnosing BV) was 75.6% and 81.8%. The Affirm assay had a sensitivity and specificity of 86.7% and 60.6% for BV, while the Aptima BV IVD assay showed sensitivities and specificities of 84.4% and 86.3%. According to the study, of the three molecular assays that were evaluated, "Aptima BV IVD demonstrated the highest specificity, which may reflect value for the *A. vaginae* target unique to that assay." The study also noted that "although assays that incorporate more bacterial targets are attractive since they reflect the bacterial diversity that has been reported in BV, it is uncertain whether they will provide better diagnostic accuracy to offset the higher cost usually charged for additional targets" (Richter et al., 2019).

One population health population study initiated by Kong et al. (2021) noted that molecular testing is both a sensitive and specific approach to testing and also a welcome tool for providers using labor-intensive traditional practices. The authors address the issue of poor compliance by providers with established gold standard guidelines such as the Amsel criteria, as well as a varied and divergent approaches to office diagnostics. The widespread availability of molecular testing could help accomplish the diagnosis of vaginitis in a single visit. The authors conclude that "compared to CE, molecular tests offer high sensitivity and specificity that provide a precise treatment route. In addition to improved accuracy, recent evidence demonstrates that the combination of sensitive and specific laboratory testing as well as careful patient evaluation have the potential to reduce unnecessary follow-up visits and improve patient care" (Kong et al., 2021).

Guidelines and Recommendations

Centers for Disease Control and Prevention (CDC)

The CDC published updated guidelines for diseases characterized by vulvovaginal itching, burning, irritation, odor or discharge in their Sexually Transmitted Infections Treatment Guidelines, 2021 (CDC, 2021b). These guidelines state that "obtaining a medical history alone has been reported to be insufficient for accurate diagnosis of vaginitis and can lead to inappropriate administration of medication…. Therefore, a careful history, examination, and laboratory testing to determine the etiology of any vaginal symptoms are warranted. Information regarding sexual behaviors and practices, sex of sex partners, menses, vaginal hygiene practices (e.g., douching), and self-treatment with oral and intravaginal medications or other products should be elicited" (CDC, 2021b).

The CDC notes that "in the clinician's office, the cause of vaginal symptoms can often be determined by pH, a potassium hydroxide (KOH) test, and microscopic examination of a wet mount of fresh samples of vaginal discharge." However, the guidelines conclude that "in settings where pH paper, KOH, and microscopy are unavailable, a broad range of clinical laboratory tests … can be used" (CDC, 2021b).

For the evaluation of BV, the CDC recommends that "BV can be diagnosed by the use of clinical criteria (i.e., Amsel's Diagnostic Criteria) or by determining the Nugent score from a vaginal Gram stain"(CDC, 2021a). Additional tests are available: "The Osom BV Blue test (Diagnostics) detects vaginal sialidase activity. The Affirm VP III (Becton Dickinson) is an oligonucleotide probe test that detects high concentrations of G. vaginalis nucleic acids (>5 x 105 CFU of G. vaginalis/mL of vaginal fluid) for diagnosing BV, *Candida* species, and *T. vaginalis*. This test has been reported to be most useful for symptomatic women in conjunction with vaginal pH measurement and presence of amine odor. . . Finally, the FemExam Test Card (Cooper Surgical) measures vaginal pH, presence of trimethylamine (a metabolic by-product of *G. vaginalis*), and proline aminopeptidase. . . This test has primarily been studied in resource-poor settings, and although it has been reported to be beneficial compared with syndromic management, it is not a preferred diagnostic method for BV diagnosis"(CDC, 2021a). The guidelines also state that due to insufficient evidence, "routine screening for BV among asymptomatic pregnant women at high or low risk for preterm delivery for preventing preterm birth is not recommended,"(CDC, 2021a), which is in compliance with the 2008 USPSTF recommendations and endorsed by the AAFP (USPSTF, 2008).

Regarding NAATs for BV, the CDC states that "BV NAATs should be used among symptomatic women only (e.g., women with vaginal discharge, odor, or itch) because their accuracy is not well defined for asymptomatic women. Despite the availability of BV NAATs, traditional methods of BV diagnosis, including the Amsel criteria, Nugent score, and the Affirm VP III assay, remain useful for diagnosing symptomatic BV because of their lower cost and ability to provide a rapid diagnosis. Culture of *G. vaginalis* is not recommended as a diagnostic tool because it is not specific. Cervical Pap tests have no clinical utility for diagnosing BV because of their low sensitivity and specificity" (CDC, 2021a).

The CDC provides information on multiple BV NAATs that are available and notes that "these tests are based on detection of specific bacterial nucleic acids and have high sensitivity and specificity for BV (i.e., *G. vaginalis, A. vaginae*, BVAB2, or *Megasphaera* type 1) and certain lactobacilli (i.e., *Lactobacillus crispatus, Lactobacillus jensenii*, and *Lactobacillus gasseri*). They can be performed on either clinician- or self-collected vaginal specimens with results available in <24 hours, depending on the availability of the molecular diagnostic platform. Five quantitative multiplex PCR assays are available: Max Vaginal Panel (Becton Dickinson), Aptima BV (Hologic), *NuSwab*® VG (LabCorp), *OneSwab*® BV Panel PCR with Lactobacillus Profiling by qPCR (Medical Diagnostic Laboratories), and *SureSwab*® BV (Quest Diagnostics). Two of these assays are FDA cleared (BD Max Vaginal Panel and Aptima BV), and the other three are laboratory-developed tests. The Max Vaginal Panel provides results by an algorithmic analysis of molecular DNA detection of Lactobacillus species (*L. crispatus* and *L. jensenii*) in addition to *G.*

vaginalis, A. vaginae, BVAB2, and *Megasphaera* type 1. This test has 90.5% sensitivity and 85.8% specificity for BV diagnosis, compared with Amsel criteria and Nugent score. It also provides results for Candida species and T. vaginalis. The Aptima BV detects *G. vaginalis, A. vaginae*, and certain *Lactobacillus* species including *L. crispatus, L. jensenii, and L. gasseri*, with sensitivity and specificity ranging from 95.0% to 97.3% and 85.8% to 89.6%, respectively (using either clinician- or patient-collected vaginal swabs). The three laboratory-developed tests (*NuSwab® VG, OneSwab®* BV Panel PCR with Lactobacillus Profiling by qPCR, and *SureSwab®* BV) have to be internally validated before use for patient care yet have good sensitivity and specificity, similar to FDA-cleared assays" (CDC, 2021a).

For the evaluation of vulvovaginal candidiasis, the CDC recommends: "Examination of a wet mount with KOH preparation should be performed for all women with symptoms or signs of VVC, and women with a positive result should be treated. For those with negative wet mounts but existing signs or symptoms, vaginal cultures for *Candida* should be considered" (CDC, 2021c). The most current guidelines for VVC diagnosis state that "vaginal culture or PCR should be obtained from women with complicated VVC to confirm clinical diagnosis and identify non–*albicans Candida*" (CDC, 2021c).

For the evaluation of trichomoniasis, the CDC recommends: "Diagnostic testing for *T. vaginalis* should be performed for women seeking care for vaginal discharge… Wet-mount microscopy traditionally has been used as the preferred diagnostic test for T. vaginalis among women because it is inexpensive and can be performed at the POC; however, it has low sensitivity (44%–68%) compared with culture. . . More highly sensitive and specific molecular diagnostic options are available, which should be used in conjunction with a negative wet mount when possible. NAATs are highly sensitive, detecting more *T. vaginalis* infections than wet-mount microscopy among women. . . The *OSOM®* trichomonas rapid test (Diagnostics) is an antigen-detection test that uses immunochromatographic capillary flow dipstick technology that can be performed at the POC by using clinician-obtained vaginal specimens. Results are available in approximately 10–15 minutes, with sensitivities of 82%–95% and specificity of 97%–100%, compared with wet mount, culture, and transcription-mediated amplification . . . The Solana trichomonas assay (Quidel) is another rapid test for the qualitative detection of *T. vaginalis* DNA and can yield results <40 minutes after specimen collection. . . The Amplivue trichomonas assay (Quidel) is another rapid test providing qualitative detection of *T. vaginalis* that has been FDA cleared for vaginal specimens from symptomatic and asymptomatic women"(CDC, 2022) and "the Affirm VP III (Becton Dickinson) is an oligonucleotide probe test that detects high concentrations of G. vaginalis nucleic acids (>5 x 105 CFU of G. vaginalis/mL of vaginal fluid) for diagnosing BV, *Candida* species, and *T. vaginalis*. This test has been reported to be most useful for symptomatic women in conjunction with vaginal pH measurement and presence of amine odor (sensitivity of 97%); specificity is 81% compared with Nugent" (CDC, 2021a).

In the updated Sexually Transmitted Infections Treatment Guidelines, the CDC also mentions the FDA-cleared Aptima *T. vaginalis* assay that may be used for detection of *T. vaginalis* from symptomatic or asymptomatic women (CDC, 2022).

American Academy of Family Physicians (AAFP)

The AAFP published an article (Hainer & Gibson, 2011) on the diagnosis of vaginitis which states that: "Physicians traditionally diagnose vaginitis using the combination of symptoms, physical examination, pH of vaginal fluid, microscopy, and the whiff test. When combined, these tests have a sensitivity and specificity of 81 and 70 percent, respectively, for BV; 84 and 85 percent for vulvovaginal candidiasis; and 85 and 100 percent for trichomoniasis when compared with the DNA probe standard…A cost-effectiveness analysis of diagnostic strategies for vaginitis undiagnosed by pelvic examination, wet-mount preparation, and related office tests showed that the least expensive strategy was to perform

yeast culture, gonorrhea and chlamydia probes at the initial visit, and Gram stain and *Trichomonas* culture only when the vaginal pH exceeded 4.9. Other strategies cost more and increased duration of symptoms by up to 1.3 days" (Hainer & Gibson, 2011).

In 2018, the AAFP published the following guidelines:

- "Symptoms alone cannot differentiate between the causes of vaginitis. Office-based or laboratory testing should be used with the history and physical examination findings to make the diagnosis. (C evidence rating)
- Do not obtain culture for the diagnosis of bacterial vaginosis because it represents a polymicrobial infection. (C evidence rating)
- Nucleic acid amplification testing is recommended for the diagnosis of trichomoniasis in symptomatic or high-risk women. (C evidence rating)" (Paladine & Desai, 2018).

U.S. Preventive Services Task Force Recommendations (USPSTF)

In 2020, the USPSTF published recommendations discouraging the use of screening for BV in pregnancy: "The USPSTF recommends against screening for bacterial vaginosis in pregnant persons not at increased risk for preterm delivery". On a similar note, the USPSTF maintains its 2008 recommendation stating "that the current evidence is insufficient to assess the balance of benefits and harms of screening for bacterial vaginosis in pregnant persons at increased risk for preterm delivery" (Owens et al., 2020).

American College of Obstetrics and Gynecology (ACOG)

The ACOG published in 2020 Practice Bulletin Number 215 on vaginitis in nonpregnant patients. These guidelines were reaffirmed in 2022. In these guidelines, the ACOG made these recommendations for diagnostic testing based on good and consistent scientific evidence (Level A):

- "The use of Amsel clinical criteria or Gram stain with Nugent scoring is recommended for the diagnosis of bacterial vaginosis."
- "Nucleic acid amplification testing is recommended for the diagnosis of trichomoniasis."
- "In a symptomatic patient, diagnosis of vulvovaginal candidiasis requires one of the following two findings: 1) visualization of spores, pseudohyphae, or hyphae on wet-mount microscopy or 2) vaginal fungal culture or commercial diagnostic test results positive for *Candida* species."

The ACOG also published recommendations based on limited or inconsistent scientific evidence (Level B), along with a series of recommendations based on consensus and expert opinion (Level C). Those relating to diagnostic testing are reported below:

- "Patients should be retested within 3 months after treatment for *T vaginalis* because of the high rates of infection recurrence" (Level B).
- "Pap tests are not reliable for the diagnosis of vaginitis. Diagnostic confirmation is recommended for incidental findings of vulvovaginal candidiasis, bacterial vaginosis, or trichomoniasis on a Pap test" (Level B).
- "A complete medical history, physical examination of the vulva and vagina, and clinical testing of vaginal discharge (i.e. pH testing, a potassium hydroxide [KOH] "whiff test", and microscopy) are recommended for the initial evaluation of patients with vaginitis symptoms" (Level C).

The ACOG mentions in Bulletin Number 215 that an advanced single-swab panel test that combines multiplex PCR and DNA probe technology could be a promising alternative to microscopy for BV, trichomoniasis, and candidiasis (ACOG, 2020).

Infectious Diseases Society of America (IDSA) Clinical Practice Guidelines

The IDSA has published an updated clinical guideline (Pappas et al., 2016) for the management of candidiasis in which recommendations include diagnosing vulvovaginal candidiasis before proceeding with empiric antifungal therapy. The usual diagnosis is clinical based on signs and symptoms of vaginitis such as pruritus, irritation, vaginal soreness, vulvar edema, erythema and many others. Clinical signs and symptoms are nonspecific and could be attributed to causes other than vulvovaginal candidiasis. Therefore, authors recommend confirming clinical diagnosis by a wet -mount preparation with saline and 10% KOH to demonstrate the presence of yeast and a normal pH. In cases where signs and symptoms are suggestive of vulvovaginal candidiasis, but microscopic findings and pH are negative, culture testing confirms the diagnosis according to published guidelines. The IDSA also discusses the possible use of PCR in diagnosing invasive candidiasis, even though the guidelines later state that "Cultures of blood or other samples collected under sterile conditions have long been considered diagnostic gold standards for invasive candidiasis…The role of PCR in testing samples other than blood is not established" (Pappas et al., 2016).

In the 2018 IDSA *A Guide to Utilization of the Microbiology Laboratory for Diagnosis of Infectious Diseases*, the IDSA states, "For vaginosis (altered vaginal flora) a Gram stain and recently available microbiome-based assays are more specific than culture and probe testing for *Gardnerella vaginalis* alone… A number of point-of-care tests can be performed from a vaginal discharge specimen while the patient is in the healthcare setting. Although point-of-care tests are popular, the sensitivity and specificity for making a specific diagnosis vary widely and these assays, while rapid, are often diagnostically poor (Miller et al., 2018)." The IDSA notes that the FDA has approved the use of the Max Vaginal Panel by Becton Dickinson in symptomatic females. "Preliminary data show greater specificity of this approach compared to methods that identify only *G. vaginalis*, as well as consistency in both reproducible as well as standardized results" (Miller et al., 2018).

Society of Obstetricians and Gynecologists of Canada (SOGC)

The SOGC published guidelines for the screening and management of BV in pregnancy. These guidelines state that the following:

- "In symptomatic pregnant women, testing for and treatment of bacterial vaginosis is recommended for symptom resolution. Diagnostic criteria are the same for pregnant and non-pregnant women (I-A).
- Asymptomatic women and women without identified risk factors for preterm birth should not undergo routine screening for or treatment of bacterial vaginosis (I-B).
- Women at increased risk for preterm birth may benefit from routine screening for and treatment of bacterial vaginosis (I-B).
- Testing should be repeated one month after treatment to ensure that cure was achieved (III-L)" (Yudin & Money, 2017).

The SOGC also published guidelines regarding the screening and management of trichomoniasis, VVC, and BV. These guidelines state that "Bacterial vaginosis should be diagnosed using either clinical

(Amsel's) or laboratory (Gram stain with objective scoring system) criteria (II-2A)" (van Schalkwyk & Yudin, 2015).

Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website https://www.cms.gov/medicare-coverage-database/search.aspx . For the most up-to-date Medicaid policies and coverage, please visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

On October 28, 2016, the FDA approved an automatic class III designation for the BD MAX™ Vaginal Panel (FDA, 2016). Following the initial approval, an additional 510(k) Substantial Equivalence Determination Decision Summary was released on October 21, 2019, with the following note: "Routine post market surveillance activities informed BD of an unanticipated high rate of nonreportable result rate for the BD MAX Vaginal Panel. Through investigations, BD identified four design modifications intended to improve the tolerance of the BD MAX Vaginal Panel without significantly impacting the validated clinical and analytical performance. . . One of the four design modifications was determined to be significant with the potential to affect the safety or effectiveness of the device and is the focus of this submission. The cumulative changes require minor modifications to the labeling" (FDA, 2019b).

On May 23, 2019, the FDA approved the use of the Aptima® BV Assay for the detection and identification of bacterial vaginosis. According to the FDA, "the Aptima BV assay is an in vitro nucleic acid amplification test that utilizes real time transcription-mediated amplification (Schwebke et al., 2020) for detection and quantitation of ribosomal RNA from bacteria associated with bacterial vaginosis (BV), including Lactobacillus (*L. gasseri, L. crispatus*, and L. *jensenii*), *Gardnerella vaginalis*, and *Atopobium vaginae*. The assay reports a qualitative result for BV and does not report results for individual organisms. The assay is intended to aid in the diagnosis of BV on the automated Panther system using clinician-collected and patient-collected vaginal swab specimens from females with a clinical presentation consistent with vaginitis and/or vaginosis" (FDA, 2019a).

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
81513	Infectious disease, bacterial vaginosis, quantitative real-time amplification of RNA markers for Atopobium vaginae, Gardnerella vaginalis, and Lactobacillus species, utilizing vaginal-fluid specimens, algorithm reported as a positive or negative result for bacterial vaginosis Proprietary test: Aptima® BV Assay Lab/Manufacturer: Hologic, Inc

81514	Infectious disease, bacterial vaginosis and vaginitis, quantitative real-time amplification of DNA markers for Gardnerella vaginalis, Atopobium vaginae, Megasphaera type 1, Bacterial Vaginosis Associated Bacteria-2 (BVAB-2), and Lactobacillus species (L. crispatus and L. jensenii), utilizing vaginal-fluid specimens, algorithm reported as a positive or negative for high likelihood of bacterial vaginosis, includes separate detection of Trichomonas vaginalis and/or Candida species (C. albicans, C. tropicalis, C. parapsilosis, C. dubliniensis), Candida glabrata, Candida krusei, when reported (Do not report 81514 in conjunction with 87480, 87481, 87482, 87510, 87511, 87512, 87660, 87661) Proprietary test: BD MAX™ Vaginal Panel Lab/Manufacturer: Becton Dickson and Company
82120	Amines, vaginal fluid, qualitative
83986	pH; body fluid, not otherwise specified
87070	Culture, bacterial; any other source except urine, blood or stool, aerobic, with isolation and presumptive identification of isolates
87149	Culture, typing; identification by nucleic acid (DNA or RNA) probe, direct probe technique, per culture or isolate, each organism probed
87150	Culture, typing; identification by nucleic acid (DNA or RNA) probe, amplified probe technique, per culture or isolate, each organism probed
87210	Smear, primary source with interpretation; wet mount for infectious agents (eg, saline, India ink, KOH preps)
87480	Infectious agent detection by nucleic acid (DNA or RNA); Candida species, direct probe technique
87481	Infectious agent detection by nucleic acid (DNA or RNA); Candida species, amplified probe technique
87482	Infectious agent detection by nucleic acid (DNA or RNA); Candida species, quantification
87510	Infectious agent detection by nucleic acid (DNA or RNA); Gardnerella vaginalis, direct probe technique
87511	Infectious agent detection by nucleic acid (DNA or RNA); Gardnerella vaginalis, amplified probe technique
87512	Infectious agent detection by nucleic acid (DNA or RNA); Gardnerella vaginalis, quantification
87660	Infectious agent detection by nucleic acid (DNA or RNA); Trichomonas vaginalis, direct probe technique
87661	Infectious agent detection by nucleic acid (DNA or RNA); Trichomonas vaginalis, amplified probe technique
87797	Infectious agent detection by nucleic acid (DNA or RNA), not otherwise specified; direct probe technique, each organism
87798	Infectious agent detection by nucleic acid (DNA or RNA), not otherwise specified; amplified probe technique, each organism
87799	Infectious agent detection by nucleic acid (DNA or RNA), not otherwise specified; quantification, each organism
87800	Infectious agent detection by nucleic acid (DNA or RNA), multiple organisms; direct probe(s) technique
87801	Infectious agent detection by nucleic acid (DNA or RNA), multiple organisms; amplified probe(s) technique
87808	Infectious agent antigen detection by immunoassay with direct optical (ie, visual) observation; Trichomonas vaginalis

87905	Infectious agent enzymatic activity other than virus (eg, sialidase activity in vaginal fluid)
0330U	Infectious agent detection by nucleic acid (DNA or RNA), vaginal pathogen panel, identification of 27 organisms, amplified probe technique, vaginal swab Proprietary test: Bridge Women's Health Infectious Disease Detection Test Lab/Manufacturer: Bridge Diagnostics/ThermoFisher and Hologic Test Kit on Panther Instrument
0352U	Infectious disease (bacterial vaginosis and vaginitis), multiplex amplified probe technique, for detection of bacterial vaginosis–associated bacteria (BVAB-2, Atopobium vaginae, and Megasphera type 1), algorithm reported as detected or not detected and separate detection of Candida species (C. albicans, C. tropicalis, C. parapsilosis, C. dubliniensis), Candida glabrata/Candida krusei, and trichomonas vaginalis, vaginal-fluid specimen, each result reported as detected or not detected Protietary test: Xpert® Xpress MVP Lab/Manufacturer: Cepheid®
Q0111	Wet mounts, including preparations of vaginal, cervical or skin specimens

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

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Revision History

Revision Date	Summary of Changes
03/06/2024	Reviewed and Updated: Updated the background, guidelines and recommendations, and evidence-based scientific references. Literature review did not necessitate any modifications to coverage criteria.
12/06/2023	Off-cycle Review, no updates outside of the coverage criteria: Following discussion with our clinical advisory board (CAB) and experts in the field, the decision was made to change CC9 from DNMCC to MCC. Now reads: "9) NAAT panel testing designed to detect more than one type of vaginitis (VVC, BV, and/or trichomoniasis; e.g., BD MAX™ Vaginal Panel, NuSwab® VG, Xpert® Xpress MVP) MEETS COVERAGE CRITERIA." Title changed from "Diagnosis of Vaginitis including Multi-target PCR Testing" to "Diagnosis of Vaginitis"
05/31/2023	Off-cycle coding modification: Added CPT 0330U from G2149 to this policy, as it is a better fit.
03/01/2023	Reviewed and Updated: Updated background, guidelines, and evidence-based scientific references. Literature review necessitated the following changes in coverage criteria: All CC were edited for clarity and consistency CC8, removed "when limited to known pathogenic species" and provided clarifying language. CC8 now reads: "For individuals with symptoms of bacterial vaginosis (BV), NAAT specific to the diagnosis of BV (e.g., Aptima® BV; OneSwab® BV Panel PCR with Lactobacillus Profiling by qPCR; SureSwab® Advanced BV, TMA) and single or multitarget PCR testing for the diagnosis of BV MEETS COVERAGE CRITERIA. Addition of new CC9: "9) NAAT panel testing designed to detect more than one type of vaginitis (VVC, BV, and/or trichomoniasis; e.g., BD MAX™ Vaginal Panel, NuSwab® VG, Xpert® Xpress MVP) DOES NOT MEET COVERAGE CRITERIA." Added CPT 0352U
03/09/2022	Annual review: Updated background, guidelines and recommendations, and evidence-based scientific references. Literature review did not necessitate modification to coverage criteria
12/08/2021	Off-Cycle Review: Addition for clarity of NAAT testing to the following CC: • Nucleic Acid Amplification Test (NAAT), polymerase chain reaction (PCR) testing, and Multitarget PCR testing, when limited to known pathogenic species, **MEETS COVERAGE CRITERIA** for the diagnosis of bacterial vaginosis**.**
09/01/2021	Off-Cycle Review: Definitions, Guidelines and Recommendations and Evidence-based Scientific References were updated. Literature review necessitated following coverage criteria changes: Per CDC 2021 addition of "especially HIV" and "incarceration" to the following CC: • Screening for *Trichomonas* **MEETS COVERAGE CRITERIA** for individuals with risk factors including: new or multiple partners; history of sexually transmitted

	diseases (STDs), especially HIV; exchange of sex for payment; incarceration, or injection drug use. Per CDC 2021 change for Candida PCR from DNMCC to MCC: • Polymerase Chain Reaction (PCR) based identification of *Candida* **MEETS COVERAGE CRITERIA** for individuals with complicated vulvovaginal candidiasis (VVC) to confirm clinical diagnosis and identify non-*albicans Candida*. Per CDC 2021 change for bacterial vaginosis PCR from DNMCC to MCC • PCR testing and Multitarget polymerase chain reaction (PCR) testing for the diagnosis of bacterial vaginosis **MEETS COVERAGE CRITERIA.** Replaced "women" and "patients" with "individuals". CPT Status Change: Status change: 87481 and 87482 enforcement was changed from not covered to pa not req
03/03/2021	Annual review: Updated background, guidelines, and evidence-based scientific references. Literature review did not necessitate any modifications to coverage criteria.
09/22/2020	Off-cycle coding change: 0068U was deleted.
03/10/2020	Annual review: Updated background, guidelines, and evidence-based scientific references. Literature review did not necessitate any modifications to intent of CCs. Changed the prior E&I CCs to DNMCC and added in the statement concerning lack of published literature to precede these CCs.
03/01/2019	Annual review: Updated scientific background, guidelines, and evidence-based scientific references. Per AHS protocol, reordered the CCs so that those that MCC are listed first, followed by the CCs that DNMCC, and finally listing CCs that are E&I. Added CC stating that using molecular-based panel testing, including, but not limited to testing such as SmartJane™, to test for microorganisms involved in vaginal flora imbalance and/or infertility is **E&I**. Added CPT 87210 and PLA 0068U.
03/16/2018	Off-Cycle Review: Policy was reviewed to change the Annual Review Cycle. Literature review did not necessitate any modification to coverage criteria.
09/15/2017	Annual review: Definitions, Background, Guidelines and Recommendations and Evidence-based Scientific References were updated. CC revised to clarify and reflect 2015 CDC recommendations. CC1- Rewritten to include methodologies for testing; CC2-Rewritten for clarity; -CC3 Addition of cultures for *Candida* per CDC and IDSA, 2016; -CC4- Per CDC 2015 is as acceptable as Gram Stain and Affirm CDC; -CC5-Is the preferred test for Trichomonas per CDC (Link)and uptodate; -CC6-Per CDC, (link); -CC7-Per CDC (link); USPSTF (link); CC8-Per CDC (link); USPSTF (link); Added CPT Code 87905, changed 87481 from PA not required to not covered, added 87070 as PA not required
09/19/2016	Annual review: Literature review did not necessitate any change.
09/18/15	Initial presentation

Diagnostic Testing of Common Sexually Transmitted Infections

Policy Number: AHS – G2157 – Diagnostic Testing of Common Sexually Transmitted Infections	Prior Policy Name and Number, as applicable: Portions of this policy replaces portions of M2097- Identification of Microorganisms using Nucleic Acid Probes
Initial Presentation Date: 09/25/2018 Effective Date: 02/01/2025	

Policy Description

Sexually transmitted infections (STIs), often referred to as sexually transmitted diseases or STDs, include a variety of pathogenic bacteria, virus, and other microorganisms that are spread through sexual contact and can cause a multitude of complications if left untreated. Chlamydia and gonorrhea, caused by *Chlamydia trachomatis* and *Neisseria gonorrhoeae*, respectively, have high rates of occurrence in the United States and can cause pelvic inflammatory disease (PID), infertility, and pregnancy complications. The causative agent of syphilis is *Treponema pallidum*; if left untreated, syphilis can lead to serious cardiac and neurological conditions (Ghanem & Tuddenham, 2024). Human papillomavirus (HPV) is a double-stranded DNA virus that can be sexually transmitted and is associated with cervical cancer, vulvar/vaginal cancer, anal cancer, oropharyngeal cancer, penile cancer, and both genital and nongenital warts. "Globally,

anogenital HPV is the most common sexually transmitted infection" with an estimated 80% of sexually active adults exposed to it at least once in their lifetime (Palefsky, 2024). Herpes simplex virus (HSV) is a common STI where many individuals are asymptomatic. HSV infection has been linked to an increased risk of other infections, including human immunodeficiency virus (HIV), and in rare cases, can also result in HSV meningitis or proctitis (Albrecht, 2024). In general, risk factors for STIs can include both behavioral elements, such as multiple sex partners, working in a sex trade, and inconsistent use of condoms when in non-monogamous relationships as well as demographic risks, including men who have sex with men (MSM), prior STI diagnosis, admission to correctional facilities, and lower socioeconomic status (Ghanem & Tuddenham, 2024).

This policy is limited to testing for *C. trachomatis*, *N. gonorrhoeae*, *T. pallidum*, *T. vaginalis* (for guidance on *T. vaginalis* in vaginitis, see AHS-M2057-Diagnosis of Vaginitis Including Multi-Target PCR Testing), HSV, and HPV. The following conditions and/or tests are discussed in the corresponding policies:

- Human Immunodeficiency Virus: AHS-M2116
- Hepatitis B and C: AHS-G2036-Hepatitis Testing
- Pediatric Preventive Screening: AHS-G2042
- Cervical Cancer Screening: AHS-G2002
- Pathogen Panel Testing: AHS-G2149

For STI screening in pregnant individuals, please see AHS-G2035-Prenatal Screening (Nongenetic).

Related Policies

Policy Number	Policy Title
AHS-G2002	Cervical Cancer Screening
AHS-G2035	Prenatal Screening (Nongenetic)
AHS-G2036	Hepatitis Testing
AHS-G2042	Pediatric Preventive Screening
AHS-G2149	Pathogen Panel Testing
AHS-M2057	Diagnosis of Vaginitis
AHS-M2116	Human Immunodeficiency Virus

Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) Antibody testing for syphilis infection **MEETS COVERAGE CRITERIA** in the following situations:

a) For any asymptomatic person in a high-risk category (see Notes 1 & 2), once a year assessment using either a "standard" or "reverse" algorithm that includes initial and confirmatory tests for any initial positive test, such as:
    i) Treponemal Ig test **and**
    ii) Nontreponemal Ig test.
b) For diagnosis of any person presenting with signs and/or symptoms of a syphilis infection (see Note 3).
c) Once every three months for HIV-positive men or MSM.
d) Treponemal Ig testing and nontreponemal testing (once prior to transplant) as a part of a pre-transplant assessment in both donors and recipients of an allogeneic hematopoietic stem cell transplantation (allo-HCT).
e) When a nontreponemal test is used as a test of cure (TOC) for a positive syphilis infection.

2) For asymptomatic individuals NOT belonging to a high-risk category (see Notes 1 & 2), antibody screening for syphilis **MEETS COVERAGE CRITERIA** only in the following situations:
    a) As part of newborn screening.
    b) As part of follow-up in a victim of sexual assault.
    c) For sexually active individuals less than 18 years of age (annually).

3) Polymerase chain reaction (PCR) testing and nucleic acid amplification testing (NAAT) for syphilis **DO NOT MEET COVERAGE CRITERIA**.

4) NAAT for chlamydia **MEETS COVERAGE CRITERIA** in the following situations:
    a) Once a year assessment for any asymptomatic person in a high-risk category (see Notes 1& 4).
    b) For diagnosis of any person presenting with signs and/or symptoms of a chlamydial infection (see Note 5).
    c) For the diagnosis of any person with suspected lymphogranuloma venereum (LGV).
    d) At least three months after initial chlamydial diagnosis as a TOC.

5) For asymptomatic individuals NOT belonging to a high-risk category (see Notes 1 & 4), screening for chlamydia **MEETS COVERAGE CRITERIA** only in the following situations:

a) As part of newborn screening.

b) As part of follow-up in a victim of sexual assault.

c) For sexually active individuals less than 18 years of age (annually).

6) Serology testing for chlamydia or LGV **DOES NOT MEET COVERAGE CRITERIA**.

7) NAAT for gonorrhea **MEETS COVERAGE CRITERIA** in the following situations:

a) Once a year assessment for any asymptomatic person in a high-risk category (see Notes 1 & 4).

b) For diagnosis of any person presenting with signs and/or symptoms of a gonorrheal infection (see Note 6).

c) As a TOC for treatment.

8) For an individual that does not respond to initial treatment, culture testing for *N. gonorrhoeae* to determine antimicrobial susceptibility **MEETS COVERAGE CRITERIA**.

9) For asymptomatic individuals NOT belonging to a high-risk category (see Notes 1 & 4), screening for gonorrhea **MEETS COVERAGE CRITERIA** only in the following situations:

a) As part of newborn screening.

b) As part of follow-up in a victim of sexual assault.

c) For sexually active individuals less than 18 years of age (annually).

10) NAATs or PCR-based testing for *T. vaginalis* **MEETS COVERAGE CRITERIA** in the following situations:

a) Symptomatic individuals (see Note 7).

b) Asymptomatic individuals belonging to a high-risk group:

i) Concurrent STI or history of STIs.

ii) Individuals in high prevalence settings, such as STI clinics.

iii) Individuals who exchange sex for payment.

11) Rapid identification of Trichomonas by enzyme immunoassay **DOES NOT MEET COVERAGE CRITERIA**.

12) For symptomatic individuals (see Note 8), testing for *Mycoplasma genitalium* using NAAT **MEETS COVERAGE CRITERIA**.

13) For asymptomatic individuals (see Note 8), screening for *M. genitalium* using NAAT **DOES NOT MEET COVERAGE CRITERIA**.

14) When an individual meets any of the conditions described above, multitarget PCR testing (targets limited to *C. trachomatis, N. gonorrhoeae, T. vaginalis*, and *M. genitalium*) **MEETS COVERAGE CRITERIA**.

15) For individuals with active genital ulcers or mucocutaneous lesions, nucleic acid amplification testing (NAAT) for herpes simplex virus-1 (HSV-1) or herpes simplex virus-2 (HSV-2) **MEETS COVERAGE CRITERIA**.

16) Immunoassay testing for HSV-1 and and/or herpes simplex (non-specific type test) **DOES NOT MEET COVERAGE CRITERIA**.

17) Type-specific serologic testing for HSV-2 using a glycoprotein G2 (gG2) test **MEETS COVERAGE CRITERIA** in the following situations:

    a) Recurrent or atypical genital symptoms or lesions in individuals with a negative herpes simplex virus PCR or culture result.

    b) For the clinical diagnosis of genital herpes in individuals with a negative PCR or culture result or without laboratory confirmation.

    c) When an individual's partner has genital herpes.

18) In asymptomatic individuals, screening for HSV-1 or HSV-2 **DOES NOT MEET COVERAGE CRITERIA**.

19) In the diagnosis and/or assessment of cancer or cancer therapy (immunohistochemistry testing for p16 or NAAT testing for high-risk human papillomavirus [HR-HPV]), testing for HR-HPV **MEETS COVERAGE CRITERIA**.

20) For individuals under 30 years of age, testing for HPV **DOES NOT MEET COVERAGE CRITERIA** in the following situations:

    a) To screen for oncogenic high-risk types, such as HPV-16 and HPV-18, as part of a general sexually transmitted disease (STD) or sexually transmitted infection (STI) screening process or panel for asymptomatic individuals.

    b) As part of the diagnosis of anogenital warts.

    c) To screen for low-risk types of HPV.

    d) In the general population, either as a part of a panel of tests or as an individual NAAT to determine HPV status.

21) Prior to beginning a preexposure prophylaxis (PrEP) regimen, the following screens/tests **MEET COVERAGE CRITERIA**:
    a) Serum creatinine and estimated creatinine clearance to determine baseline renal function.
    b) Antibody screening to confirm a baseline negative antibody result for HIV.
    c) Hepatitis B (HBV) and/or Hepatitis C screening to identify positive individuals.
    d) Pregnancy testing.

22) While an individual is undergoing a preexposure prophylaxis (PrEP) regimen for HIV prevention, the following screens/tests **MEET COVERAGE CRITERIA**:
    a) A blood test once every three months to confirm a negative antibody result for HIV.
    b) Serum creatinine and estimated creatinine clearance three months after beginning PrEP and up to one time every six months thereafter to assess renal function.
    c) NAAT screening, based on anatomic site of exposure, for gonorrhea and chlamydia:
        i) Once every three months for MSM and for individuals with child-bearing potential.
        ii) Nine months after PrEP is initiated and once every six months thereafter for sexually active individuals.
    d) Blood test to screen for syphilis once every three months in MSM and individuals with child-bearing potential.
        i) Once every three months for MSM and for individuals with child-bearing potential.
        ii) Nine months after PrEP is initiated and once every six months thereafter for sexually active individuals.
    e) Pregnancy testing once every three months.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

23) Nucleic acid testing to determine antimicrobial susceptibility in *N. gonorrhoeae* or macrolide resistance in *M. genitalium* **DOES NOT MEET COVERAGE CRITERIA**.

24) Using nucleic acid testing to quantify the following microorganisms **DOES NOT MEET COVERAGE CRITERIA**:

a) *Chlamydia trachomatis*

b) *Neisseria gonorrhoeae*

c) Herpes Simplex Virus-1

d) Herpes Simplex Virus-2

e) Human Papillomavirus

f) *Treponema pallidum*

NOTES:

Note 1: For sexually active children and adolescents under the age of 18, risk factors for chlamydia, gonorrhea, and/or syphilis infection as defined by the CDC include: initiating sex early in adolescence; living in detention facilities; receiving services at STD clinics; being involved in commercial sex exploitation or exchanging sex for drugs, money, food, or housing; having multiple sex partners, having sequential sex partnerships of limited duration or concurrent partnerships; failing to use barrier protection consistently and correctly; having lower socioeconomic status, and facing numerous obstacles to accessing healthcare. At-risk individuals also include: males who have sex with males (YMSM); transgender youths; youths with disabilities, substance abuse, or mental health disorders (CDC, 2021c).

Note 2: <u>High-risk for Syphilis (Cantor et al., 2016; CDC, 2023a)</u>:

- Sexually active men who have sex with men (MSM)
- Sexually active HIV-positive status
- Having a sexual partner recently diagnosed with a STI
- Exchanging sex for money or drugs
- Individuals in adult correctional facilities
- During pregnancy when the following risk factors are present:
  - Sexually active HIV-positive status
  - Sexually active with multiple partners
  - Sexually active in conjunction with drug use or transactional sex
  - Late entry to prenatal care (i.e., first visit during the second trimester or later) or no prenatal care
  - Methamphetamine or heroin use
  - Incarceration of the woman or her partner
  - Unstable housing or homelessness

Note 3: <u>Signs and Symptoms of a Syphilis Infection (CDC, 2018, 2023a)</u>

- Chancre
- Skin rash and/or mucous membrane lesions in mouth, vagina, anus, hands, and feet
- Condyloma lata
- Secondary symptomology can include fever, fatigue, sore throat, swollen lymph nodes, weight loss, muscle aches, headache, and hair loss
- Signs and symptoms of neurosyphilis can include severe headache, trouble with muscle movements, muscle weakness or paralysis (not being able to move certain parts of the body), numbness, and changes in mental status (trouble focusing, confusion, personality change) and/or dementia (problems with memory, thinking, and/or making decisions).
- Signs and symptoms of ocular syphilis can include eye pain or redness, floating spots in the field of vision ("floaters"), sensitivity to light, and changes in vision (blurry vision or even blindness).
- Signs and symptoms of otosyphilis may include hearing loss, ringing, buzzing, roaring, or hissing in the ears ("tinnitus"), balance difficulties, and dizziness or vertigo.
- Signs and symptoms of late/tertiary syphilis include inflammatory lesions of the cardiovascular system (e.g., aortitis, coronary vessel disease), skin (e.g., gummatous lesions), and bone (e.g., osteitis).

Note 4: <u>High-risk for Chlamydia and/or Gonorrhea (CDC, 2021b, 2024a, 2024d; LeFevre, 2014)</u>:

- Sexually active men who have sex with men (MSM)
- Sexually active HIV-positive status
- Sexually active women under the age of 25
- Women age 25 or over who have multiple sexual partners
- Having a sexual partner recently diagnosed with an STI
- Previous or concurrent STI
- Exchanging sex for money or drugs

Note 5: <u>Signs and Symptoms of a Chlamydia Infection (CDC, 2021b, 2024a):</u>

- Genital symptoms, including "discharge, burning during urination, unusual sores, or rash"
- Pelvic Inflammatory Disease (PID), including "symptoms of abdominal and/or pelvic pain, along with signs of cervical motion tenderness, and uterine or adnexal tenderness on examination"
- Urethritis

- Pyuria
- Dysuria
- Increase in frequency in urination
- Epididymitis (with or without symptomatic urethritis) in men
- Proctitis
- Sexually acquired chlamydial conjunctivitis

Note 6: Signs and Symptoms of Gonorrhea (CDC, 2024d):

- Dysuria
- Urethral infection
- Urethral or vaginal discharge
- Epididymitis (Testicular or scrotal pain)
- Rectal infection symptoms include anal itching, discharge, rectal bleeding, and painful bowel movements

Note 7: Signs and Symptoms of Trichomoniasis (CDC, 2023b):

- Vaginal or penile discharge
- Itching, burning sensation, or soreness of the genitalia
- Discomfort or burning sensation during/after urination and/or ejaculation
- Urethritis
- Epididymitis
- Prostatitis

Note 8: Signs and Symptoms of *M. genitalium* Infection (CDC, 2021a):

- When present, typical symptoms of *Mgen*-urethritis in men include dysuria, urethral pruritus, and purulent or mucopurulent urethral discharge
- When present, typical symptoms of *Mgen* cervicitis in women include vaginal discharge, vaginal itching, dysuria, and pelvic discomfort
- When present, typical symptoms of PID due to *Mgen* include mild to severe pelvic pain, abdominal pain, abnormal vaginal discharge, and/or bleeding

Table of Terminology

Term	Definition
AAP	American Academy of Pediatrics
AGIHO/DGHO	Infectious Diseases Working Party of the German Society for Hematology and Medical Oncology
AIDs	Acquired immune deficiency syndrome
AIN	Anal intraepithelial neoplasia
ASCUS	Atypical squamous cells of undetermined significance
BASHH	British Association for Sexual Health and HIV

BD	Becton Dickinson
CDC	Centers for Disease Control and Prevention
CI	Confidence interval
CIA	Chemiluminescence immunoassay
CIN2+	Cervical intraepithelial neoplasia grade 2+
CIN3	Cervical intraepithelial neoplasia grade 3
CLIA	Chemiluminescent assay
CLIA ’88	Clinical Laboratory Improvement Amendments of 1988
CMIA	Chemiluminescence immunoassay
CMS	Centers for Medicare and Medicaid
CNS	Central nervous system
CPS	Canadian Paediatric Society
CSF	Cerebrospinal fluid
CT	*Chlamydia trachomatis*
DAG-KBT	German Working Group for Blood and Marrow Transplantation
DFE	Darkfield examination
DNA	Deoxyribonucleic acid
DRE	Digital rectal examination
E7-MPG	E7 multiplex genotyping
EBV	Epstein Barr virus
ED	Emergency department
EIA	Enzyme immunoassay
ELISA	Enzyme-linked immunosorbent assay
FDA	Food and Drug Administration
FEMS	Federation of European Microbiological Societies
FIA	Fluorescence immunoassay
FNA	Fine needle aspiration
FTA	Fluorescent treponemal antibody
GC	Gonococcal
gG2	Glycoprotein G2
GP5+/6+	General primer 5+/6+
HBV	Hepatitis B
HC2	Hybrid capture 2
hCG	Human chorionic gonadotropin
HIV	Human immunodeficiency virus
HIV-1	Human immunodeficiency virus-1
HPV	Human papillomavirus
HPV-16	Human papillomavirus type 16
HPV-18	Human papillomavirus type 18

HR-HPV	High risk or oncogenic HPV testing
HSIL	High-grade squamous intraepithelial lesion
HSV	Herpes simplex virus
HSV-1	Herpes simplex virus-1
HSV-2	Herpes simplex virus-2
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IHC	Immunohistochemistry
IMCA	Immunochemiluminometric assay
ISH	In situ hybridization
ISVVD	The International Society for the Study of Vulvovaginal Disease
IUSTI	International Union Against Sexually Transmitted Infections
JAMA	Journal of the American Medical Association
LDTs	Laboratory-Developed Tests
LGSIL	Low grade squamous intraepithelial lesion on cytologic smear of anus
LGV	Lymphogranuloma venereum
LSIL	Low-grade squamous intraepithelial lesions
MG	*Mycoplasma genitalium*
Mgen	*Mycoplasma genitalium*
MHA-TP	Microhemagglutination Assay for *Treponema pallidum* antibodies
MLST	Multilocus sequence typing
mRNA	Messenger RNA
MSM	Men having sex with men
MTC	Male Training Center for Family Planning & Reproductive Health
NA	Not applicable
NAAT	Nucleic acid amplification testing
NCCN	National Comprehensive Cancer Network
NG	Neisseria gonorrhoeae
NGU	Nongonococcal urethritis
NICE	National Institute for Health and Care Excellence
NOS	Not otherwise specified
NTT	Nontreponemal test
ORPH-1	Oropharynx-1
OS	Overall survival
PCR	Polymerase chain reaction
PID	Pelvic inflammatory disease
POC	Point-of-care
POCT	Point-of-care test
PrEP	Preexposure prophylaxis

PWID	People who inject drugs
RFLP	Restriction fragment length polymorphism
RNA	Ribonucleic acid
RPR	Rapid plasma reagin test
SDA	Strand displacement amplification
STDs	Sexually transmitted diseases
STIs	Sexually transmitted infections
TMA	Transcription-mediated amplification
TOC	Test of cure
TPHA	*Treponema pallidum* hemagglutination
TP-IgA	*Treponema pallidum* IgA antibodies
TPPA	*Treponema pallidum* particle agglutination
TP-PA	*T. pallidum* passive particle agglutination
TT	Treponemal test
TV	Trichomonas vaginalis
USPSTF	United States Preventive Services Task Force
VDRL	Venereal disease research laboratory
VIN	Vulvar intraepithelial neoplasia

Scientific Background

Chlamydia

Chlamydia, caused by the bacterium *Chlamydia trachomatis*, is usually an asymptomatic sexually transmitted infection that can be passed to a newborn from an infected mother, potentially resulting in conjunctivitis and/or pneumonia. Symptomatic infections can include cervicitis, pelvic inflammatory disease (PID), and Fitzhugh-Curtis syndrome in women as well as epididymitis, prostatitis, and reactive arthritis triad in men. Both men and women can have proctitis, urethritis, conjunctivitis, pharyngitis, and genital lymphogranuloma venereum as a result of a chlamydial infection. Nucleic acid amplification testing (NAAT) for chlamydia is the gold standard due to high specificity and sensitivity instead of using culture testing, microscopy, or antigen detection (Hsu, 2024). In the U.S. alone, in 2022, over 1.6 million cases of chlamydia were reported to the CDC, but the CDC estimates that 2.86 million chlamydial infections occur annually (CDC, 2024a, 2024h). This under-reporting is due to individuals who are asymptomatic and, therefore, do not seek treatment. Highest prevalence occurs among men who have sex with men (MSM) and young people. “It is estimated that 1 in 20 sexually active young women aged 14-24 years has chlamydia” (CDC, 2024a).

Mycoplasma genitalium (Mgen) is a sexually transmitted infection that is strongly associated with urethritis symptoms, similar to *Chlamydia trachomatis* and *Neisseria gonorrhoeae* (Goldstein et al., 2021). *Mgen* can infect the uterus, urethra, or rectum, and causes infections in all genders. In men, common symptoms of *Mgen*-urethritis include: dysuria, urethral pruritus, and purulent or mucopurulent urethral discharge. In women, common symptoms of Mgen cervicitis include: vaginal discharge, vaginal itching, dysuria, and pelvic discomfort. The prevalence of *Mgen* in the United States is estimated to be 1.7% among people aged 14 to 59 years. However, the prevalence of *Mgen* in clinical-based populations are higher; a multicenter study around diverse geographic regions of the United States found the prevalence of *Mgen* to be 10.3% in people seeking care (CDC, 2021a).

Gonorrhea

Gonorrhea is a sexually transmitted infection caused by the bacterium *Neisseria gonorrhoeae*. A gonorrheal infection can cause many of the same complications as chlamydia, including PID, cervicitis, and Fitzhugh-Curtis syndrome in women and epididymitis in men. Urethritis, pharyngitis, and proctitis can also occur; in fact, "*N. gonorrhoeae* can be isolated from the urethra in up to 90 percent of women with gonococcal cervicitis" (Ghanem, 2024). Like chlamydia, if left untreated, gonorrhea can be spread from mother to newborn, resulting in conjunctivitis. NAAT is the best method to diagnose gonorrhea, but culture testing is still used to determine antimicrobial susceptibility due to an increase in antibiotic resistance (Unemo, 2020). In 2022, the CDC reported an 11% increase since 2018 in the number of cases of gonorrhea reported in the United States (CDC, 2024g). The CDC also reported 207,255 new cases of gonorrhea in the United States in 2018 (CDC, 2024g).

Syphilis

Syphilis is caused by the bacterium *Treponema pallidum*, and it progresses, if left untreated, through various stages—primary, secondary, early-latent, late-latent, and late-stage syphilis—until infecting the central nervous system. "Syphilis infection is associated with HIV infection and increases the risk for acquiring or spreading HIV" (Cantor et al., 2016). Worldwide, the median rates of infection in males and females were 17.7 cases per 100,000 and 17.2 cases per 100,000, respectively, according to the World Health Organization. The U.S. has reported an increase in the rate of syphilis between 2000 and 2016, and approximately 90% of the new cases of primary and secondary syphilis during this period occurred in men with 81% occurring in men who have sex with men (MSM). Of concern, there has also been an increased number of cases of syphilis in women. In 2021, 2855 cases of congenital syphilis were reported. This included 220 syphilis-related stillbirths and infant deaths (Hicks & Clement, 2023).

Similar to other STIs, syphilis is often asymptomatic. For symptomatic syphilis, the signs and symptoms can vary, depending on the stage of disease. Primary syphilis can have a characteristic chancre, a skin lesion that is usually painless and often heals even in the absence of treatment. Secondary syphilis occurs weeks to months later and can be manifested by typical immunologic responses, such as fever, lethargy, and so on; adenopathy; rash; alopecia; hepatitis; gastrointestinal abnormalities; and even early symptoms of neurological infection, if left untreated. Later stages of syphilis can include cardiovascular abnormalities and progression of neurological syphilitic infection. Asymptomatic, latent syphilis can also occur; moreover, "pregnant women with latent syphilis can transmit *T. pallidum* to their fetus for up to four years after acquisition" (Hicks & Clement, 2023).

The standard protocol for diagnosing a syphilis infection is to use a two-tiered serological testing algorithm of treponemal testing and nontreponemal testing. Treponemal testing is typically more complex than the latter, and they both rely upon the detection of specific treponemal antigens using enzyme immunoassay (EIA), particle agglutination assay, fluorescence, or chemiluminescence immunoassay (CIA). Nontreponemal testing methods, including the rapid plasma reagin test (RPR) and the venereal disease research laboratory (VDRL) test, "are based upon the reactivity of serum from infected patients to a cardiolipin-cholesterol-lecithin antigen" (Hicks & Clement, 2022). Rapid serological testing using darkfield microscopy is not as universally used due to complexity and cost. NAAT has not been FDA-approved at this time and is not typically performed for genital syphilis. "There is no internationally approved PCR for *T. pallidum* and accordingly, it is crucial to select a strictly validated method and always use it with appropriate quality controls" (Janier et al., 2014).

Herpes Simplex Virus (HSV)

Herpes Simplex Virus-2 (HSV-2) is the common cause of most of genital herpes simplex infections worldwide with the CDC estimating that 50 million people in the U.S. were infected with HSV-2 in 2015 (Workowski & Bolan, 2015). In 2018, CDC estimates show there were 572,000 new genital herpes infections in the U.S. among people aged 14 to 49; moreover, HSV-1 genital herpes has increased in recent years. This trend is believed to be due to a decline in childhood oral HSV-1 infections that in the past increased immune resistance to genital HSV-1 infections (CDC, 2024b). Primary genital herpes infections can present with genital ulcers as well as other immunological responses, such as fever and lymphadenopathy; however, for some people, a primary genital herpes infection is asymptomatic. Nonprimary infections occur when a patient acquires HSV-1 with pre-existing HSV-2 antibodies or vice versa. Recurrent infections can be either symptomatic or asymptomatic, which can be referred as subclinical. A minority of HSV-positive patients can also present with meningitis and/or proctitis

(Albrecht, 2024). Vertical transmission from mother to newborn can occur during delivery, especially if the mother acquires a primary infection near the end of the pregnancy. This vertical transmission can occur even if the mother is asymptomatic (Riley & Wald, 2022). Diagnosis of genital herpes infection can be performed by viral culture, NAAT, and serological testing. "Cell culture and PCR-based testing are the preferred tests for a patient presenting with active lesions, although PCR-based testing has the greatest overall sensitivity and specificity" (Albrecht, 2024).

Human Papillomavirus (HPV)

Anogenital HPV infection is the most common STI worldwide with an estimation that "almost all sexually active individuals will acquire HPV at some point in their lifetime" (Palefsky, 2024). This is due to the large number of different types of HPV known to infect the genital tract—at least 40 characterized to date—and the transitory nature of HPV infections. HPV is associated with a variety of cancers, including anal, penile, vulvar, vaginal, and oropharyngeal cancer; moreover, the carcinogenic effect of an HPV infection can be years after the initial diagnosis of HPV. Multiple HPV vaccinations have been approved for use in the U.S., and the CDC recommends vaccination for HPV for all children ages 11 or 12 (CDC, 2024c). HPV can be detected from swab samples and can be included in many routine cervical exams. High-risk oncogenic HPV testing is commercially available (Feldman & Crum, 2024).

HIV Preexposure Prophylaxis (PrEP)

An estimated 1.1 million people in the United States currently live with human immunodeficiency virus (HIV). HIV is a virus that, while treatable, does not have a cure and results in serious health consequences that may include acquiring acquired immune deficiency syndrome (AIDs). In the 2019 issue of JAMA, the U.S. Preventive Services Task Force updated guidelines on recommendations for HIV screening and preventive services. The USPSTF reviewed the evidence regarding Preexposure prophylaxis (PrEP), which is the use of antiretroviral medication to prevent HIV infection ang provided a grade A recommendation for PrEP in certain circumstances (CDC, 2022; USPSTF, 2019). The USPSTF determined that PrEP is "of substantial benefit in decreasing the risk of HIV infection in persons at high risk of HIV acquisition" (USPSTF, 2019). As a preventive medication, PrEP involves a single treatment taken orally with "combined tenofovir disoproxil fumarate and emtricitabine," or tenofovir disoproxil fumarate alone, which can be considered as an alternative regimen (USPSTF, 2019). In addition, adherence to PrEP is "highly associated with its efficacy in preventing the acquisition of HIV infection; thus, adherence to PrEP is central in realizing its benefit." Overall, the guidance is to provide PrEP with antiretroviral therapy to persons at high risk of HIV acquisition (USPSTF, 2019).

To determine status for PrEP provision, the CDC recommends antigen/antibody testing to confirm that patients do not currently have HIV infection. At a minimum providers should test to confirm a negative antibody result within a week before initiating (or re-initiating) PrEP regimens (CDC, 2022). There are a few ways to accomplish HIV testing: "(1) drawing blood and sending the specimen to a laboratory for testing or (2) performing a rapid, point-of-care FDA-approved fingerstick blood test. Oral rapid tests should not be used to screen for HIV infection when considering PrEP use because they can be less sensitive than blood tests" (CDC, 2022).

The PrEP regimen may cause decreases in renal function. Usually, these are of small or limited clinical significance, but occasional cases of acute renal failure have been documented. The CDC guidance indicates that all patients who are considered for PrEP should have renal function assessed during the beginning of treatment. Other screenings recommended before PrEP initiation include a screening for HBV.

The following table for PrEP testing recommendations for clinicians was compiled by the CDC (CDC, 2022):

Provide the following services:	**Screening tests/samples**
At 3 months after PrEP initiation:	• Test for HIV. • Measure serum creatinine and estimate creatinine clearance. • Provide medication adherence and behavioral risk reduction support. • Additionally, for o MSM: screen for bacterial STIs*; o Women with reproductive potential: test for pregnancy; and o PWID: assess access to sterile needles/syringes and to drug treatment services.
Every 3 months after the first 3-month follow-up	• Test for HIV. • Provide medication adherence and behavioral risk reduction support. • Additionally, for o MSM: screen for bacterial STIs*; o Women with reproductive potential: test for pregnancy; and o PWID: assess access to sterile needles/syringes and to substance use disorder treatment services.

Every 6 months after the first 3-month follow-up	• Measure serum creatinine and estimate creatinine clearance. • For all sexually active patients: Screen for bacterial STIs*.

*Nucleic Acid Amplification Test (NAAT) to screen for gonorrhea and chlamydia based on anatomic site of exposure; blood test for syphilis.

Proprietary Testing

BD Onclarity HPV Assay

The BD Onclarity HPV Assay, a qualitative in vitro assay of cervical swabs using PCR (i.e., a nucleic acid amplification test or NAAT), is offered by Becton, Dickinson and Company and is approved by the FDA. This test specifically identifies types 16, 18 and 45, while concurrently detecting the other high-risk (HR) HPV types (including 31, 51, 52, 33/58, 35/39/68, and 56/59/66). For HR-HPV 31, 51, 52, 33/58, 35/39/68, and 56/59/66, this is "the only FDA-approved assay to individually identify and report these genotype results" (BD, 2020).

Becton, Dickinson and Company note that "the BD Onclarity HPV Assay is indicated: 1) In women 21 years and older with ASC-US (atypical squamous cells of undetermined significance) cervical cytology test results, the BD Onclarity HPV Assay can be used to determine the need for referral to colposcopy; 2) In women 21 years and older with ASC-US cervical cytology test results, the BD Onclarity HPV Assay can be used to detect high-risk HPV genotypes 16, 18 and 45. This information together with physicians assessment of screening history, other risk factors, and professional guidelines, may be used to guide patient management. The results of this test are not intended to prevent women from proceeding to colposcopy; 3) In women 30 years and older, the BD Onclarity HPV Assay can be used together with cervical cytology to adjunctively screen to detect high risk HPV types. This information, together with the physicians assessment of screening history, other factors, and professional guidelines, may be used to guide patient management; 4) In women 30 years and older, the BD Onclarity HPV Assay can be used to detect high-risk HPV genotypes 16, 18 and 45. This information, together with the physicians assessment of screening history, other factors, and professional guidelines, may be used to guide patient management; and 5) In women 25 years and older, the BD Onclarity HPV Assay can be used as a first-line primary cervical cancer screening test to detect high risk HPV, including 16 and 18. Women who test negative for the high risk HPV types by the BD Onclarity HPV Assay should be followed up in accordance with the physicians assessment of screening and medical history, other risk factors, and professional guidelines. Women who test positive for HPV genotypes 16 and/or 18 by the BD Onclarity HPV Assay should be

referred to colposcopy. Women who test high risk HPV positive and 16 and 18 negative by the BD Onclarity HPV Assay (12 other HR HPV Positive) should be evaluated by cervical cytology to determine the need for referral to colposcopy" (FDA, 2021).

Cepheid Xpert® CT/NG

Cepheid offers the Cepheid Xpert® CT/NG test, an FDA-approved nucleic acid amplification test to detect *Chlamydia trachomatis* (CT) and/or *Neisseria gonorrhoeae* (NG) using urogenital specimens and extragenital specimens (pharynx and rectum))(FDA, 2012a, 2019a). It is performed using the GeneXpert® Instrument Systems with a qualitative *in vitro* real-time PCR "for the automated detection and differentiation of genomic DNA from Chlamydia trachomatis (CT) and/or Neisseria gonorrhoeae (NG)" (FDA, 2012b, 2019b) and is stated to provide results for up to 96 specimens in approximately 90 minutes (Cepheid, 2022). The assay may be used to "test the following specimens from asymptomatic and symptomatic individuals: female and male urine, patient-collected vaginal swabs (collected in a clinical setting), clinician-collected endocervical swabs, and female and male pharyngeal and rectal swabs" (Cepheid, 2022). Sensitivity and specificity of this test are dependent on the manner in which samples were collected, with patient collected vaginal swab, endocervical swab, urine, and pharyngeal swab specimens showing sensitivity and specificity in the mid to high ninetieth percentile. Rectal swab specimens showed a lower sensitivity for both CT (86%) and NG (91.2%), but specificity in the 99th percentile, similar to the specificity of the other sample collection methods (Cepheid, 2022).

Abbott Alinity™ m STI Assay

Abbott offers the Alinity™ m STI AMP Kit. The test is "an in vitro reverse transcription-polymerase chain reaction (RT-PCR) assay for the direct, qualitative detection and differentiation of RNA from Chlamydia trachomatis (CT), Trichomonas vaginalis (TV), Mycoplasma genitalium (MG), and DNA from Neisseria gonorrhoeae (NG)." The test is a four in one multiplex assay that detects four reactions. The first result should appear in under 115 minutes. Abbott reports a sensitivity of 100% for all analytes and specificity with "no cross-activity observed with 148 organisms." The assay may be used to test the following specimens: "endocervical swab specimens, clinician-collected vaginal swab specimens, self-collected vaginal swab specimens (in a clinical setting), gynecological specimens collected in ThinPrep PreservCyt solution, female urine, and male urine" (Abbott, 2023).

Goldstein et al. (2021) performed an international, multicenter study to evaluate accuracy, reproducibility, and clinical performance of the Alinity™ m STI assay. The

Alinity™ m STI assay was compared with commonly used STI assays. "The Alinity m STI assay identified accurately and precisely single and mixed pathogens from an analytical panel of specimens" and had "high overall agreement rates with comparator STI assays" (Goldstein et al., 2021).

Analytical Validity

A 2005 study by Cook and colleagues (Cook et al., 2005) reviewed the validity of NAAT for chlamydia and gonorrhea from urine samples as compared to swabs obtained directly from either the cervix or urethra. They reviewed 29 different studies and only included studies using collections of samples obtained from two anatomic sites. Each test required either a secondary culture confirmation or a secondary NAAT-based confirmation. Over 20,000 different patients were included in the pooled study, and three different NAAT assays were monitored—polymerase chain reaction (PCR), transcription-mediated amplification (Golden et al.), and strand displacement amplification (SDA). "The pooled study specificities of each of the 3 assays exceeded 97% when urine samples were tested, for both chlamydial infection and gonorrhea and in both men and women." The use of PCR for gonorrheal testing, though, from female urine samples had only 55.6% specificity. The authors concluded the following: "Results of nucleic acid amplification tests for *C. trachomatis* on urine samples are nearly identical to those obtained on samples collected directly from the cervix or urethra. Although all 3 assays can also be used to test for *N. gonorrhoeae*, the sensitivity of the polymerase chain reaction assay in women is too low to recommend its routine use to test for gonorrhea in urine specimens" (Cook et al., 2005).

Due to an increase in demand for enzyme immunoassay-based testing of syphilis, Wong et al. (2011) evaluated the validity of such testing—using the Trep-Sure EIA test—to that of the documented Venereal Disease Research Laboratory (VDRL) test and Treponema pallidum particle agglutination (TPPA) assay. Their research included 674 samples. The EIA-based test had a sensitivity of 98.0% and a specificity of 98.6% (Cantor et al., 2016). The authors conclude that "an IgM/IgG sensitive EIA would be an effective alternative to VDRL for syphilis screening" (Wong et al., 2011). An earlier study using another EIA-based assay, the Trep-Check IgG EIA test, conducted at the National Microbiology Laboratory of Canada (Tsang et al., 2007) did not report as positive results as the Wong study. This research consisted of 604 samples submitted from local or provincial hospitals for confirmation of local testing. Their findings were that the Trep-Check IgG EIA had a sensitivity of 85.3% and specificity of 95.6%, but they also report a positive predictive value of 53.7% (Tsang et al., 2007) as compared to the positive predictive value of 98.4% of the Trep-Sure EIA test (Cantor et al., 2016; Wong et al., 2011). These results can be compared to the published results of the accuracy of the TPPA assay of 87.1% sensitivity, 100% specificity, and 100% positive predictive

value—albeit in a smaller sample size (n = 198) (Cantor et al., 2016; Juarez-Figueroa et al., 2007).

The U.S. Preventive Services Task Force (USPSTF) conducted a systematic review of the use of serologic screening for genital herpes and published their findings in 2016 (USPSTF, 2023). Their extensive review consisted of 17 different studies, ranging from 24 to 3,290 participants, in 19 different publications. Reviewing only the serological testing of HSV-2, they note that the "pooled estimates of sensitivity and specificity of the most commonly used test at the manufacturer's cutpoint were 99% (95% CI, 97%-100%) and 81% (95% CI, 68%-98%), respectively." However, they also note that "use of this test at the manufacturer's cutpoint in a population of 100 000 with a prevalence of HSV-2 of 16% (the seroprevalence in US adults with unknown symptom status) would result in 15 840 true-positive results and 15,960 false-positive results (positive predictive value, 50%)." They note the potential psychosocial harm due to false-positive results. The authors conclude, "Serologic screening for genital herpes is associate with a high rate of false-positive test results and psychosocial harms" (USPSTF, 2023).

In 2021, the US Preventive Services Task Force issued a brief update on genital herpes simplex diagnostics. Their assessment found that viral culture continues to be the gold standard for HSV infections. For central nervous system infections of HSV, PCR continues to be the gold standard, because of the assay's sensitivity of 80% to 90% for lesion specimens. They also indicated that serological tests are used to detect previous infections of herpes simplex in asymptomatic patients, specifying the Western blot assay as the most validated method. In addition, they noted: "two type-specific glycoprotein G serological tests are commercially available in the United States. Sensitivity and specificity of these tests are comparable to the Western blot assay" (Glass, 2021).The ATHENA study conducted in 2008-2009 and published in Lancet in 2011 consisted of more than 40,000 women in the U.S. aged 25 or over in 61 different clinical centers. The goal was to assess high-risk HPV16 and HPV18 testing versus traditional methods. Their results show that "in women who had colposcopy, the Cobas HPV test was more sensitive than liquid-based cytology for detection of CIN3 [cervical intraepithelial neoplasia grade 3] or worse" with 92.0% versus 53.3% for liquid cytology. "Addition of liquid-based cytology to HPV testing increased sensitivity for CIN3 or worse to 96.7%...but increased the number of screen positives by 35.2%." The authors conclude, "HPV testing with separate HPV16 and HPV18 detection could provide an alternative, more sensitive, and efficient strategy for cervical cancer screening than do methods based solely on cytology" (Castle et al., 2011). Guenat and colleagues report a coefficient of variation of less than 8% for repeatability and reproducibility when using the Novaprep HQ+ medium in liquid-based cytology for HPV (Guenat et al., 2016). Another study comparing the validity of using urine samples

in comparison with cervical samples for monitoring HPV in women over the age of 30 shows that the sensitivity of the urine testing varies considerably depending on the NAAT assay used. The multiplex type-specific PCR (E7-MPG) assay had a sensitivity of 80% and specificity of only 61% whereas the GP5+/6+ PCR assay resulted in 58% and 89%, respectively, for sensitivity and specificity as compared to the gold standard cervical swabs (Tshomo et al., 2017).

A study by Golden et al. (2019) compared the sensitivity of syphilis serological testing using the rapid plasma reagin (RPR) test and an experimental 23S rRNA *Treponema pallidum* real-time transcription-mediated amplification (Golden et al.) assay. This study included 545 men who have sex with men (MSM); a total of 506 pharyngeal specimens and 410 rectal specimens were provided for this study. Twenty-two men were diagnosed with syphilis based on serological testing results; further, two more men were diagnosed based on TMA testing results. The authors report that "At least 1 specimen was TMA positive for 12 of 24 men with syphilis (sensitivity, 50% [95% confidence interval [CI], 29 to 71%]). RPR testing and clinical diagnosis were 92% sensitive (95% CI, 73 to 99%) in identifying infected men" (Golden et al., 2019). A combinatory approach of mucosal TMA testing and serological testing may improve the sensitivity of syphilis screening.

Pham et al. (2020) reported on a new prototype point-of-care test (POCT) based on detecting IgA antibodies for *Treponema pallidum* (TP-IgA), which is a new biomarker for active syphilis. Using "458 pre-characterised stored plasma in China… and 503 venous blood samples collected from pregnant/postpartum in South Africa," the performance of the POCT was compared against TPHA and RPR tests. In the sub-study group from China, the index test had a sensitivity of 96.1% (95% confidence interval 91.7%-98.5%) and specificity of 84.7% (95% confidence interval 80.1%-88.6%) for "identification of active syphilis," (TPHA positive, RPR positive) and identified 71% samples of past-treated syphilis, defined as a TPHA positive but RPR negative test. In the sub-study group from South Africa, the index test had a 100% sensitivity (95% confidence interval 59%-100%) for active syphilis, and "correctly identified all nine women with past syphilis." The researchers cite that in comparison to other POCTs on the market, this new test can "identify past syphilis whilst maintaining a high sensitivity for active syphilis infections," and "support[s] the global effort in prevention of mother to child transmission and elimination of congenital syphilis in settings where laboratory capacity is limited" (Pham et al., 2020).

In 2019, (Bristow et al.) compared the use of the Xpert® CT/NG test on extragenital samples to the already FDA-approved APTIMA transcription mediated amplification Combo 2 assay. They found the Xpert® CT/NG test performed similarly, but with a faster turnaround time and increased potential for same-day treatment. Their results

demonstrated that "the pooled positive and negative percent agreement for detection of CT in rectal specimens was 89.72% (95% CI: 84.97%, 93.64%) and 99.23% (95% CI: 98.74%, 99.60%), and in pharyngeal specimens, they were 89.96% (95% CI: 66.38%, 99.72%) and 99.62% (95% CI: 98.95%, 99.95%) respectively. For NG detection in rectal specimens, the pooled positive and negative per cent agreement was 92.75% (95% CI: 87.91%, 96.46%) and 99.75% (95% CI: 99.46%, 99.93%), and in pharyngeal specimens, they were 92.51% (95% CI: 85.84%, 97.18%) and 98.56% (95% CI: 97.69%, 99.23%) respectively" (Bristow et al., 2019).

A separate study done earlier by Cosentino et al. (2017) also compared APTIMA's transcription mediated Combo 2 assay with the Xpert® CT/NG assay and found that "For *C. trachomatis*, neither system was >95% sensitive from the rectum, though both were >99.5% specific. For *N. gonorrhoeae*, Xpert had higher sensitivity than Aptima, but with more false positives from pharyngeal samples."

Clinical Validity and Utility

A 2017 review of POCTs versus near-patient NAAT for chlamydia reviewed 11 different studies consisting of a combined total of more than 13,000 patients. The pooled results show that POCTs have a sensitivity of only 53%, 37%, and 63% for cervical swabs, vaginal swabs, and male urine, respectively, but that the specificity for each ranged from 97-99%. The near-patient NAAT has a sensitivity of >98% regardless of sample with a specificity of 99.4%. "The systematic reviews show that antigen detection POCTs for CT [*C. trachomatis*], although easy to use, lacked sufficient sensitivity to be recommended as a screening test. A near-patient NAAT shows acceptable performance as a screening or diagnostic test but requires electricity, takes 90 min and is costly" (Kelly et al., 2017). Likewise, a review of five POCTs and one near-patient NAAT for gonorrhea in 2017 show that POTC immunochromatographic tests and optical immunoassays had sensitivities ranging from 12.5% to 70% compared to laboratory NAAT for cervical and vaginal swab samples. The specificities of the near-patient NAATs were >99.8% with sensitivities >95% (Guy et al., 2017).

A 2018 review of laboratory testing for *T. pallidum* in Australia (Brischetto et al., 2018) compared the clinical value of PCR testing for syphilis as compared to the traditional serological testing using RPR, agglutination, and/or chemiluminescence immunoassay (CMIA). This review covered all testing at the Australian lab from 2010 to 2017. They show that 19% of PCR results were positive for syphilis with 97% of those patients also showing positive serological results. The *T. pallidum* PCR had a sensitivity of 68% and specificity of 99% as compared to the serology testing sensitivity of 97% and 88% specificity. "Our results show that most patients with positive *T. pallidum* PCR results also had positive syphilis serology. Therefore, *T. pallidum* PCR adds little clinical value

over serology for the diagnosis of syphilis in certain clinical settings" (Brischetto et al., 2018). A 2015 Chinese study (Zhiyan et al., 2015) does show that the CMIA screening is not as specific as the TPPA agglutination assay for syphilis with 18 of the 149 CMIA-positive samples being false-positive results.

The 2016 USPSTF review of genital herpes serological testing (USPSTF, 2023) included a review of the HerpeSelect serological test consisting of the data from ten studies with a combined total of 6537 participants. The pooled, combined results show a sensitivity of 99% and specificity of 81%. Four additional studies they reviewed used the biokit HSV-2 Rapid Test assay. These studies had a combined total of 1512 participants. The sensitivity is considerably lower (84%), but the specificity was higher than the HerpeSelect assay (95%).

A study by Liu and associates (Liu et al., 2014) evaluated the clinical performance of the QuantiVirus HPV E6/E7 mRNA with respect to identifying ≥Grade 2 cervical intraepithelial neoplasia. Approximately 40.3% of the 335 female patients tested positive for high-risk HPV. They note that "the positivity rate of HPV E6/E7 mRNA increased with the severity of cytological and histological evaluation…a high specificity and a low positivity rate of E6/E7 mRNA testing as a triage test in HPV DNA-positive women can be translated into a low referral for colposcopy" (Liu et al., 2014). Another study of the QuantiVirus system in 2017 (Yao et al., 2017) of 404 HPV-positive women show no statistical difference between QuantiVirus and cytological testing in sensitivity, specificity, positive predictive value, and negative predictive value for predicting high-grade squamous intraepithelial lesion (HSIL). "HPV E6/E7 mRNA detection in cervical exfoliated cells shows the same performance as Pap triage for HSIL identification for HPV-positive women. Detection of HPV E6/E7 mRNA may be used as a new triage option for HPV-positive women" (Yao et al., 2017). A review by Arbyn and colleagues concerning the efficacy of repeat cytology versus HPV testing for atypical squamous cells of undetermined significance (ASCUS) and low-grade squamous intraepithelial lesions (LSIL) demonstrated that the pooled sensitivity of the Hybrid Capture 2 (HC2) assay for the high-risk HPV types was significantly higher than performing repeat cytology (relative sensitivity of 1.27 and 1.23, respectively) for detecting CIN2+ but was significantly lower than repeat cytology for LSIL. "HPV-triage with HC2 can be recommended to triage women with ASCUS because it has higher accuracy…than repeat cytology. When triaging women with LSIL, an HC2 test yields a significantly higher sensitivity, but a significantly lower specificity, compared to repeat cytology. Therefore, practice recommendations for management of women with LSIL should be balanced, taking local circumstances into account" (Arbyn et al., 2013).

A study by Gaydos et al. (2019) showed that, for women in the emergency department (ED), the use of rapid diagnostic tests for *Chlamydia trachomatis* and *Neisseria*

gonorrhoeae infections can improve clinical management. This randomized clinical trial was composed of 254 women undergoing pelvic examinations for both *C. trachomatis* and *N. gonorrhoeae* testing; the women were split into control and rapid test groups. For the rapid test group, the GeneXpert rapid test was used. The authors report that "Undertreatment for both *C trachomatis* and *N gonorrhoeae* in the ED was 0% for the rapid test group and 43.8% for the control standard-of-care group. Clinicians overtreated 46.5% of uninfected standard-of-care control patients for *C trachomatis* compared with 23.1% of uninfected rapid test patients. For patients uninfected with *N gonorrhoeae*, clinicians overtreated 46.7% of standard-of-care control patients compared with 25.4% of rapid test patients" (Gaydos et al., 2019). These results show that rapid testing of *C. trachomatis* and *N. gonorrhoeae* led to a significant reduction in overtreatment compared to the control group.

Guidelines and Recommendations

National Comprehensive Cancer Network (NCCN)

Anal Carcinoma (NCCN, 2023a): HPV, especially high-risk types HPV-16 and HPV-18, are linked to anal carcinoma. The NCCN refers to a study that detected HPV in 84% of anal carcinoma samples and 0% in rectal cancer samples, and they state that "the prevalence of HPV-16/18 to be 72% in patients with invasive anal cancer." Precursor high-grade anal intraepithelial neoplasia (Marcell & Health) "can be identified by cytology, HPV testing, digital rectal examination, high-resolution anoscopy, and/or biopsy." They also state that "data suggest that HPV- and/or p16-positivity are prognostic for improved OS [overall survival] in patients with anal carcinoma." For females, the NCCN also recommends a gynecologic examination, including cervical cancer screening, due to the link between HPV and anal carcinoma.

Cervical Cancer (NCCN, 2024a): "Persistent human papillomavirus (HPV) infection is the most important factor in the development of cervical cancer. The incidence of cervical cancer appears to be related to the prevalence of HPV in the population…. Screening methods using HPV testing may increase detection of adenocarcinoma." The NCCN lists chronic, persistent HPV infection along with persistently abnormal Pap tests as criteria to be considered for women contemplating hysterectomy after the completion of child-bearing.

Head and Neck Cancers (NCCN, 2024b): The NCCN in the Head and Neck Cancers guidelines now specifically states, "Tumor human papillomavirus (HPV) testing by p16 immunohistochemistry (IHC) required" in their workup for cancer of the oropharynx because the p16 status dictates the treatment options to be considered (per the ORPH-1 workup). This version of the guidelines also includes a page on the "Principles

of P16 Testing for HPV-Mediated Oropharyngeal Cancer" where they state the following:

- "P16 expression correlates with HPV status in geographic regions where HPV is etiologically responsible for a high proportion of cancers. Confirmatory HPV direct testing is recommended, especially for clinical trials. Clinical centers are recommended to ascertain concordance rate of p16 and direct HPV testing, as this may vary by region, if considering use of p16 IHC alone as a surrogate.
- Distinguishing p16+ patients by HPV tumor status informs prognosis. Patients with p16+ and HPV+ tumors have an improved prognosis compared to patients with p16+ and HPV-negative tumors.
- Direct HPV confirmatory tests include polymerase chain reaction (PCR) and RNA in situ hybridization (ISH).
- PCR may provide additional sensitivity while ISH provides increased specificity.
- Sufficient pathologic material for HPV testing can be obtained through FNA.
- A small proportion of tumors at non-oropharyngeal sites (eg, paranasal sinus, oral cavity, larynx) are HPV-related. However, given the small proportion and lack of consistent evidence in support of prognostic significance, routine HPV testing or p16 testing of non-oropharyngeal cancers is not recommended.
- Guidelines for testing are available from the College of American Pathologists.
- When using p16, the 70% cutoff with nuclear and cytoplasmic expression with at least moderate to strong intensity is recommended."

Occult Primary Cancers (NCCN, 2024d): The NCCN now lists HPV to be tested for Occult Primary cancers. The NCCN also states that for squamous cell carcinoma with a clinical presentation in the head and neck nodes, "Check results of p16 immunohistochemistry/human papillomavirus (HPV) in situ hybridization (ISH) and Epstein-Barr virus (EBV) (ISH); positive results can help localize primary site." Further, the guidelines note that HPV can be used as a potential immunohistochemistry marker for unknown primary cancers, including tumors identified in the cervix, vulva, vagina, penis, anal, oropharynx; a nuclear (DNA ISH) or nuclear/cytoplasmic (RNA ISH) staining pattern is recommended (NCCN, 2024d).

Penile Cancer (NCCN, 2023b): "Overall, approximately 45% to 80% of penile cancers are related to HPV, with a strong correlation with types 16, 6 and 18." Discerning whether a penile cancer lesion is infected with HPV is important for laser ablation therapy as noted in the section titled "Principles of Penile Organ-Sparing Approaches."

Vulvar Cancer (NCCN, 2024c): "Risk factors for the development of vulvar neoplasia include increasing age, infection with human papillomavirus (HPV), cigarette smoking, inflammatory conditions affecting the vulva, and immunodeficiency…. Usual-type VIN

[vulvar intraepithelial neoplasia] was linked to persistent infection with carcinogenic strains of HPV, while differentiated VIN was commonly associated with vulvar dermatologic conditions such as lichen sclerosus. In 2015, the ISVVD updated the description to three classes of vulvar lesions: 1) low-grade squamous intraepithelial lesion (LSIL) due to flat condyloma or HPV effect; 2) high-grade squamous intraepithelial lesions (HSIL, formerly considered usual-type VIN); and 3) differentiated VIN." The NCCN notes that 80-90% of HSIL cases have HPV infections, and that between 30%-69% of all vulvar cancers are believed to be "attributable to HPV infection." In the "Diagnosis and Workup" section, they state, "Appropriate patients should receive smoking cessation counseling, cervical HPV testing, and cytology testing." The guidelines also note for the surveillance of vulvar cancer: "Annual cervical/vaginal cytology tests, which may include HPV testing, can be considered as indicated for detection of lower genital tract dysplasia, although its value in detecting recurrent cancers is limited and the likelihood of detecting asymptomatic recurrence is low." (NCCN, 2024c).

U.S. Preventive Services Task Force (USPSTF)

Screening for Chlamydia and Gonorrhea (Davidson et al., 2021): The USPSTF recommends (Grade B) to screen for chlamydia and gonorrhea in "sexually active females aged 24 years or younger and in women 25 years or older who are at increased risk for infection." They also conclude (an "I" statement) "that the current evidence is insufficient to assess the balance of benefits and harms of screening for chlamydia and gonorrhea in men." Besides age, "women 25 years or older are at increased risk for infection if they have a new sex partner, more than 1 sex partner, a sex partner with concurrent partners, or a sex partner who has an STI; practice inconsistent condom use when not in a mutually monogamous relationship; or have a previous or coexisting STI. Exchanging sex for money or drugs and history of incarceration also are associated with increased risk." They clearly state that both chlamydia and gonorrhea should be tested using NAATs.

Screening for Oral Cancer (Moyer, 2014): Given the link between HPV infection and oral cancers, the USPSTF released their findings concerning the screening of asymptomatic patients. "The USPSTF concludes that the current evidence is insufficient to assess the balance of benefits and harms of screening for oral cancer in asymptomatic adults." They also state the following: "Although there is interest in screening for oral HPV infection, medical and dental organizations do not recommend it. Currently, no screening test for oral HPV infection has been approved by the U.S. Food and Drug Administration (FDA). Evaluating the accuracy of tests that detect oral HPV infection is a potentially promising area of research" (Moyer, 2014).

Serological Screening for Genital Herpes (USPSTF, 2023): HSV-2 is the primary causative agent of genital herpes, and HSV-2 infection during pregnancy can cause fetal morbidity and mortality. Due to its prevalence in the U.S. and the possible consequences of a genital herpes infection, the USPSTF researched the validity and practicality of HSV-2 screening in asymptomatic patients. They conclude that "serologic screening for genital herpes is associated with a high rate of false-positive test results and potential psychosocial harms. Evidence from RCTs [randomized clinical trials] does not establish whether preventive antiviral medication for asymptomatic HSV-2 infection has benefit." Overall, the USPSTF "recommends against routine serologic screening for genital herpes simplex virus infection in asymptomatic adolescents and adults, including pregnant persons."

Screening for Syphilis (Cantor et al., 2016): Previously, in 2004, the USPSTF "recommended routine screening for syphilis in asymptomatic men and nonpregnant women at increased risk of infection (A recommendation) and recommended against routine screening for those not at increased risk (D recommendation)." The previous study did not address the frequency of repeat testing. The current 2016 study adds to the previous recommendations. "Screening HIV-positive men or MSM for syphilis every 3-months is associated with improved syphilis detection. Treponemal or nontreponemal tests are accurate screening tests but require confirmation. Research is needed on the effect of screening on clinical outcomes; effective screening strategies, including reverse sequence screening, in various patient populations; and harms of screening."

Centers for Disease Control and Prevention (CDC)

Diseases Characterized by Genital, Anal, or Perianal Ulcers: "…all persons who have genital, anal, or perianal ulcers should be evaluated; . . . Specific evaluation of genital, anal, or perianal ulcers includes syphilis serology tests and darkfield examination from lesion exudate or tissue, or NAAT if available; NAAT or culture for genital herpes type 1 or 2; and serologic testing for type-specific HSV antibody. In settings where chancroid is prevalent, a NAAT or culture for Haemophilus ducreyi should be performed." Later, in the section specifically focused on genital HSV infections, the CDC states, "Both type-specific virologic and type-specific serologic tests for HSV should be available in clinical settings that provide care to persons with or at risk for STIs." They stress that the patient's prognosis does depend on the type of HSV infection, especially since "recurrences and subclinical shedding are much more frequent for genital HSV-2 infection than for genital HSV-1 infection." Regarding testing, "HSV NAAT assays are the most sensitive tests because they detect HSV from genital ulcers or other mucocutaneous lesions; these tests are increasingly available" (CDC, 2021b). NAATs are more sensitive than viral culture testing. On the CDC's detailed fact sheet about genital

herpes, they state, "Routine serologic HSV screening of pregnant women is not recommended" (CDC, 2024b).

In guidance on serology, the CDC states in 2021 that "type-specific HSV-2 serologic assays for diagnosing HSV-2 are useful in the following scenarios: recurrent or atypical genital symptoms or lesions with a negative HSV PCR or culture result, clinical diagnosis of genital herpes without laboratory confirmation, and a patient's partner has genital herpes. HSV-2 serologic screening among the general population is not recommended. Patients who are at higher risk for infection (e.g., those presenting for an STI evaluation, especially for persons with ≥10 lifetime sex partners, and persons with HIV infection) might need to be assessed for a history of genital herpes symptoms, followed by type-specific HSV serologic assays to diagnose genital herpes for those with genital symptoms" (CDC, 2021b).

Syphilis: Darkfield examinations and molecular tests for detecting *T. pallidum* lesion cells, fluid, or tissue are the gold standard methods for diagnosing early syphilis and congenital syphilis. According to the CDC, "Although no *T. pallidum* direct detection molecular NAATs are commercially available, certain laboratories provide locally developed and validated PCR tests for detecting *T. pallidum* DNA. A presumptive diagnosis of syphilis requires use of two laboratory serologic tests: a nontreponemal test (i.e., Venereal Disease Research Laboratory [VDRL] or rapid plasma reagin [RPR] test) and a treponemal test (i.e., the T. pallidum passive particle agglutination [TP-PA] assay, various EIAs, chemiluminescence immunoassays [CIAs] and immunoblots, or rapid treponemal assays) … Use of only one type of serologic test (nontreponemal or treponemal) is insufficient for diagnosis and can result in false-negative results among persons tested during primary syphilis and false-positive results among persons without syphilis or previously treated syphilis." If a patient shows signs and symptoms of neurosyphilis, including "cranial nerve dysfunction, auditory or ophthalmic abnormalities, meningitis, stroke, acute or chronic altered mental status, and loss of vibration sense," further testing is required-CSF cell count or protein and a reactive CSF-VDRL (CDC, 2021b).

The CDC states the signs and symptoms of neurosyphilis can include severe headache, trouble with muscle movements, muscle weakness or paralysis (not being able to move certain parts of the body), numbness, and changes in mental status (trouble focusing, confusion, personality change) and/or dementia (problems with memory, thinking, and/or making decisions). The CDC states that signs and symptoms of ocular syphilis can include eye pain or redness, floating spots in the field of vision ("floaters"), sensitivity to light, and changes in vision (blurry vision or even blindness). Lastly, the CDC states that signs and symptoms of otosyphilis may include hearing loss, ringing,

buzzing, roaring, or hissing in the ears ("tinnitus"), balance difficulties, and dizziness or vertigo" (CDC, 2023d).

"Patients who receive a diagnosis of syphilis and have neurologic, ocular, and/or otologic symptoms should be evaluated for neurosyphilis, ocular syphilis, or otosyphilis according to their clinical presentation. Patients who have syphilis and symptoms or signs suggestive of neurologic disease (e.g., cranial nerve dysfunction, meningitis, stroke, acute or chronic altered mental status, or motor or sensory deficits) should have an evaluation that includes CSF analysis before treatment. Patients with syphilis who have symptoms or signs of ocular syphilis (e.g., uveitis, iritis, neuroretinitis, or optic neuritis) should have a full ocular slit-lamp and ophthalmologic examination, including a thorough cranial nerve evaluation; if cranial nerve dysfunction is present, CSF examination is indicated" (CDC, 2024f). The CDC also recommends that, prior to donating, prospective hematopoietic stem cell transplant donors should be tested for syphilis (Dykewicz et al., 2000).

Chlamydial Infections: "Annual screening of all sexually active women aged <25 years is recommended, as is screening of older women at increased risk for infection (e.g., those who have a new sex partner, more than one sex partner, a sex partner with concurrent partners, or a sex partner who has a sexually transmitted infection…screening of sexually active young men should be considered in clinical settings with a high prevalence of chlamydia (e.g., adolescent clinics, correctional facilities, or STD specialty clinics) or for populations with a high burden of infection (e.g., MSM)" (CDC, 2021b).

NAAT testing of first-catch urine or swab specimens is recommended. In the diagnostic considerations section of chlamydial infections, the CDC does not address any differences between symptomatic or asymptomatic screening, and they do not mention any specific diagnostic considerations of patients showing signs or symptoms of a chlamydial infection. In the 2014 CDC guide for laboratory testing of chlamydia and gonorrhea, they, too, recommend using NAATs and not the older nonculture or non-NAAT testing methods. For extragenital infections such as rectal and oropharyngeal infections, the CDC recommends testing at the anatomic exposure site. NAATs demonstrate improved sensitivity and specificity in comparison to culture for extragenital infection. In addition, newly available molecular point-of-care (POC) tests for asymptomatic persons can help with faster, more efficient treatment. With symptomatic cases these POC tests can also "optimize treatment by limiting unnecessary presumptive treatment at the time of clinical decision-making and improve antimicrobial stewardship. Thus, using a POC test will likely be a cost-effective diagnostic strategy for *C. trachomatis* infection… newer NAAT-based POC tests have promising performance and are becoming commercially available" (CDC, 2021b).

Gonococcal Infections: The CDC recommendation concerning gonococcal screening is similar to that of chlamydia—sexually active women aged <25 years and older women and men in high-risk categories. "Screening for gonorrhea in men and older women who are at low risk for infection is not recommended" (CDC, 2021b). For testing genitourinary infection with N. gonorrhoeae, "culture, NAAT, and POC NAAT, such as GeneXpert (Cepheid), are available." NAAT allows for best testing of genitourinary infection.

Gonorrhea has developed resistance to nearly all the antibiotics used for its treatment, creating a need for research into identifying genetic mutations in the pathogen that are contributing to the antibiotic resistance. However, according to the CDC "currently, there is no well-studied, reliable technology that allows for antibiotic susceptibility testing from nonculture specimens. Increased laboratory culture capacity is needed" (CDC, 2024e). CDC recommends that all state and local health department labs maintain or develop the capacity to perform gonorrhea culture, or form partnerships with experienced laboratories that can perform this type of testing.

For rectal, oropharyngeal, and conjunctival infections, culture is available. The CDC states that "NAATs and POC NAATs allow for the widest variety of FDA-cleared specimen types, including endocervical and vaginal swabs and urine for women, urethral swabs and urine for men, and rectal swabs and pharyngeal swabs for men and women. However, product inserts for each NAAT manufacturer should be consulted carefully because collection methods and specimen types vary. Certain NAATs that have been demonstrated to detect commensal Neisseria species might have comparable low specificity when testing oropharyngeal specimens for N. gonorrhoeae. NAAT sensitivity for detecting N. gonorrhoeae from urogenital and nongenital anatomic sites is superior to culture but varies by NAAT type. NAAT testing of rectal and/or oropharyngeal swab specimens can be performed in certain laboratories that have met CLIA requirements even though the testing methodology has not been FDA-approved" (CDC, 2021b). Follow-up testing post-treatment for urogenital or rectal gonorrhea is not necessary, but NAAT testing should be performed 14 days after treatment for pharyngeal gonorrhea. Vaginitis is the most common symptom of infection in preadolescent girls" (Workowski & Bolan, 2015).

In the 2014 laboratory guide, the CDC states that "*N. gonorrhoeae* culture capacity is still needed for evaluating suspected cases of treatment failure and monitoring antimicrobial susceptibility." They also state, "*C. trachomatis* and *N. gonorrhoeae* culture capacity might still be needed in instances of child sexual assault in boys and extragenital infections in girls" (Papp et al., 2014).

Mycoplasma genitalium Infections: The CDC recommends that men with recurrent nongonococcal urethritis (NGU) should be tested for *M. genitalium* using an FDA-cleared NAAT. The CDC also recommends that women with recurrent cervicitis should be tested for *M. genitalium,* while testing should be considered in women with PID. For both, resistance testing is recommended if testing is available. The CDC notes that screening of asymptomatic "*M. genitalium* infection among women and men or extragenital testing for *M. genitalium* is not recommended. In clinical practice, if testing is unavailable, *M. genitalium* should be suspected in cases of persistent or recurrent urethritis or cervicitis and considered for PID" (CDC, 2021a).

"*M. genitalium* is an extremely slow-growing organism. Culture can take up to 6 months, and technical laboratory capacity is limited to research settings. NAAT for *M. genitalium* is FDA cleared for use with urine and urethral, penile meatal, endocervical, and vaginal swab samples . . . Molecular tests for macrolide (i.e., azithromycin) or quinolone (i.e., moxifloxacin) resistance markers are not commercially available in the United States. However, molecular assays that incorporate detection of mutations associated with macrolide resistance are under evaluation" (CDC, 2021a). The CDC then goes on to add, if available, men with recurrent NGU should be tested for *M. genitalium* using an FDA-cleared NAAT, resistance testing should be performed, and the results used to guide therapy. Women with recurrent cervicitis should be tested for *M. genitalium*, and testing should be considered among women with PID. Testing should be accompanied with resistance testing (CDC, 2021a).

Human Papillomavirus Infections: Even though testing for oncogenic HPV variants exists, the CDC states, "These tests should not be used for male partners of women with HPV or women aged <25 years, for diagnosis of genital warts, or as a general STI test." For patients showing signs and symptoms of anogenital warts, the CDC states, "HPV testing is not recommended for anogenital wart diagnosis, because test results are not confirmatory and do not guide genital wart management." For cervical screening, "For persons aged 30–65 years, a cytology test every 3 years, an HPV test alone every 5 years, or a cytology test plus an HPV test (cotest) every 5 years is recommended" (CDC, 2021b).

The CDC (2024c) also notes that "Routine screening for women aged 21 to 65 years old can prevent cervical cancer"; further, "There are HPV tests that can be used to screen for cervical cancer. Healthcare providers only use these tests for screening in women aged 30 years and older. HPV tests are not recommended to screen men, adolescents, or women under the age of 30 years."

Finally, the CDC (2019) states that "there is currently no approved test for HPV in men. CDC does not recommend routine testing (also called 'screening') for HPV in men. CDC

also does not recommend routine testing for diseases from HPV before there are signs or symptoms in men. Some healthcare providers offer anal Pap tests to men who may be at greater risk for anal cancer. This includes men with HIV or men who receive anal sex. If you have symptoms and are concerned about cancer, please see a healthcare provider."

International Union Against Sexually Transmitted Infections (IUSTI)

The Management of Anogenital Warts (European): "HPV detection or typing does not influence management and is not recommended. Some practitioners use the acetic acid test to diagnose sub-clinical HPV lesions; its place in diagnosis and management is uncertain" (Gilson et al., 2020).

The Diagnosis and Treatment of Gonorrhea in Adults (Unemo, 2020) NAATs, bacterial culture, and microscopy can be used in the diagnosis of uncomplicated gonorrhea. "No test offers 100% sensitivity and specificity." They do state (with a grade C recommendation) that microscopy can be used for testing symptomatic men, but it is not recommended for use in asymptomatic men, rectal infection, or endocervical infection due to low sensitivity. Culture testing is the only method to use for determining antimicrobial susceptibility, but culture testing is not as sensitive as NAAT. For NAAT-based point-of-care tests (POCTs), the guideline says: "several NAAT-based POCTs with high sensitivity and specificity are in late development." The IUSTI includes the following list for "Indications for testing" (grade C recommendation):

- Symptoms or signs of urethral discharge in men;
- Vaginal discharge with risk factor for STI (age <30 years, new sexual partner);
- Mucopurulent cervicitis;
- Persons diagnosed with any other STI;
- Sexual partner of persons with an STI or PID;
- Acute epididymo-orchitis in a male aged <40 years;
- Acute pelvic inflammatory disease;
- When screening young adults (<25 years of age) for sexually transmitted infections;
- When screening individuals with new or multiple recent sexual partners;
- Purulent conjunctivitis in a neonate or adult;
- Mother of a newborn with ophthalmia neonatorum
- Unplanned termination of pregnancy in places or populations of high gonorrhoea prevalence
- When intrauterine interventions are performed in areas of high gonorrhoea prevalence

The Management of Lymphogranuloma Venereum (de Vries et al., 2019): Lymphogranuloma venereum (LGV) is a condition caused by chlamydia. The clinical features can vary, depending on the site of inoculation (genital versus rectum) and can include hemorrhagic proctitis, lymphadenopathy, papule or pustule formation, and buboes. Reactive inflammatory responses or physical signs of in infection may include "constitutional symptoms such as low-grade fever, chills, malaise, myalgia, [and] arthralgia." Regarding a diagnosis of lymphogranuloma venereum (LGV), "a sample tested *C. trachomatis* positive with a commercial nucleic acid amplification test (NAAT) platform should be confirmed with an LGV discriminatory NAAT." Further, "For sensitive and specific detection of LGV genovar (L1, L2 and L3, including subvariant)-specific *C. trachomatis* DNA, laboratories are currently recommended to use a two-step procedure (1,B):

- "A commercially available NAAT is used to detect *C. trachomatis* DNA/RNA in suspected clinical samples. These tests cannot discriminate between LGV and non-LGV genovars. Although no commercially available *C. trachomatis* NAATs are FDA-cleared for extragenital specimens, for several NAATs sufficient evidence supports the use of these tests for the detection of C. trachomatis DNA/RNA also in rectal and pharyngeal *C. trachomatis* infections. Some *C. trachomatis* NAAT are CE-labelled for use on rectal and pharyngeal samples in Europe.
- If *C. trachomatis* DNA/RNA is detected, LGV genovar specific *C. trachomatis* DNA should be detected from the same specimen. There are multiplex NAATs for genital ulcerative disease that detect LGV but these have not yet been appropriately evaluated in the context of rectal LGV. Different in-house or laboratory-developed NAATs have been designed and used. The sensitivities of these NAATs are generally lower than the commercially available *C. trachomatis* screening NAAT" (de Vries et al., 2019).

The Management of Syphilis (Janier et al., 2014; Janier et al., 2020): The three stages (primary, secondary, and tertiary) can be overlapping. Primary syphilis begins with appearance of an ulcer (also known as a chancre), usually in the anogenital region with regional lymphadenopathy. "Any anogenital ulcer should be considered syphilitic unless proven otherwise." The secondary stage is characterized by "multisystem involvement due to bacteriaemia, within the first year but may recur up into the second year after infection" and can include skin rash, generalized lymphadenopathy, arthritis, hepatitis, splenomegaly, and kidney dysfunction. Early neurosyphilis can occur in secondary syphilis and can include "meningitis, cranial nerve palsies, auricular and ophthalmic abnormalities (such as uveitis, retinitis, otitis and papillar oedema)." They list the following as conditions of tertiary syphilis:

- "Gummatous syphilis: nodules/plaques or ulcers (skin, mucosae, visceral)"

- "Late neurosyphilis encompasses meningitis, cranial nerve dysfunction, meningovascular syphilis (stroke, myelitis) and parenchymatous neurosyphilis (general paresis, tabes dorsalis)"
- "Cardiovascular syphilis: aortic regurgitation, stenosis of coronary ostia, aortic aneurysm (mainly thoracic)"

The following guidelines were given regarding laboratory testing for *T. pallidum:*

- "Direct detection methods provide definitive diagnosis of syphilis.
- Darkfield examination (DFE) of chancres and erosive cutaneous lesions was the old gold standard method for definitive diagnosis. It gives immediate results. However, the method is labor intensive, subjective, and can result in some false positive and (many) false negative results. Due to the availability of more sensitive and specific tests (specifically the PCR), it is not recommended for routine diagnosis anymore.
- Polymerase chain reaction (PCR) testing is the preferred method particularly but not exclusively for oral and other lesions where contamination with commensal treponemes is likely. It can be performed using tissues, cerebrospinal fluid (CSF) or blood (although insensitive in the latter). There is no internationally approved PCR assay for *T. pallidum* and accordingly, it is crucial to select a strictly validated and quality-assured method and always use it with appropriate quality controls.
- Immunohistochemistry using a polyclonal antibody against *T. pallidum* can be efficient to identify treponemes in skin, mucosal and tissue lesions, but it is not suitable for routine diagnosis.
- Hybridization in tissues is not used for routine diagnosis.
- Warthin-Starry (argentic) staining on tissues is very difficult to perform and of limited value in most cases.
- (Direct fluorescent antibody test is obsolete)
- For molecular epidemiological typing, PCR, PCR-restriction fragment length polymorphism (RFLP) and/or DNA-sequencing (e.g. multilocus sequence typing (MLST) or whole genome sequencing) can be performed on clinical specimens. However, due to the highly conserved genome of *T. pallidum* the discriminatory ability of typing methods is in general low (Janier et al., 2020)"

Primary Screening Test(s)

- "TT [TPHA, MHA-TP, TPPA or EIA/ELISA/CLIA] – a TT-based screening algorithm, using by preference an automatized EIA/ELISA/CLIA, is used in many large, well-resourced European laboratories and is particularly suitable for automated high-throughput screening of asymptomatic populations including blood/plasma donors. The algorithm identifies persons with previous successful treatment of

syphilis as well as those with untreated syphilis. It is usually more sensitive in detecting very early syphilis compared to the use of a screening NTT. However, it can also result in a high number of false positive tests (i.e. very low positive predictive value) in low-prevalence populations.

- NTT [RPR or VDRL] – a NTT-based screening algorithm; preferably quantitative (i.e. to detect prozone phenomenon in infectious syphilis), is still recommended in some countries. In this algorithm, only active (Society) syphilis is detected, however, it has a lower sensitivity compared to using a TT as primary screening test, and in particular very early syphilis can be missed.
- TT combined with a NTT - this algorithm is particularly useful in cases where the suspicion of very early syphilis is high (recent chancre, contacts of syphilis cases etc.), because in some patients NTT may become reactive before TT" (Janier et al., 2020).

Confirmatory test(s) if any screening test is positive

- "In the case a TT being used alone as a primary screening test, if positive, a confirmatory TT of a different type is of limited value in informing treatment, but a reflex quantitative NTT (reaching at least 1:8 to 1:16 dilution) should be performed in all cases on the same serum (1, B). Although a confirmatory TT may be important for counselling, notification and may have a psychological impact, it has limited impact on treatment.69 In patients with a positive TT, a negative NTT and no suspicion of very early syphilis (no chancre), both tests should be repeated after 1 month (1, D). However, CLIA and EIA used in many European settings have suboptimal specificity, resulting in a low positive predictive value in low prevalence population. If such tests are used, additionally a reflex confirmatory test by TPHA or TPPA should be performed (1, C).
- In the case a NTT alone is used as a primary screening test, a positive test must be followed by a reflex TT on the same serum. If quantitative NTT was not initially done, the NTT should be repeated quantitatively (1, B).
- In the case both a TT and a NTT are used as primary screening tests such as (EIA/ELISA/CLIA/TPHA/TPPA plus VDRL/RPR), the NTT must be performed quantitatively (if not initially done) in case of positive or discrepant screening tests (1, B).
- The IgG-immunoblot for Treponema pallidum has no added major value to other TT. It is expensive and interpretation of undetermined immunoblot is elusive (1 to 4 bands).

The Management of Chlamydia Trachomatis Infections (Lanjouw et al., 2016): "Appropriate testing of symptomatic and asymptomatic sexually active individual is recommended to identify and treat the *C. trachomatis* infections." With a Grade A

recommendation, they recommend using NAATs that identify specific nucleic acid, either DNA or RNA) *of C. trachomatis* "due to their superior sensitivity, specificity, and speed."

The following list contains the indications for laboratory testing as recommended by the IUSTI with a Grade C recommendation (Lanjouw et al., 2016):

Indications for laboratory testing (Level of evidence IV; Grade C recommendation)

- Risk factor(s) for *C. trachomatis* infection and/or other STI (age<25 years, new sexual contact in the last year, more than one partner in the last year);
- Symptoms or signs of urethritis in men;
- Cervical or vaginal discharge with risk factor for STI;
- Acute epididymo-orchitis in a male aged <40 years or with risk factors for STI;
- Acute pelvic pain and/or symptoms or signs of PID;
- Proctitis/proctocolitis according to risk;
- Purulent conjunctivitis in a neonate or adult;
- Atypical neonatal pneumonia;
- Persons diagnosed with other STI;
- Sexual contact of persons with an STI or PID;
- Termination of pregnancy;
- Any intrauterine interventions or manipulations.

The Management of Genital Herpes (Patel et al., 2017): The principle change to the IUSTI guidelines in this recent version is that "HSV DNA detection rather than cell culture is now the gold standard for diagnosis." With a grade C recommendation, "serological testing is not routinely recommended in asymptomatic patients." They note that there are specific groups where it may be useful, including pregnant women, sexual partners of HSV-positive people, those with a history of recurrent or atypical genital disease, and those with first-episode genital herpes whose differentiation may aid in counseling and management (because seroconversion happens typically at 90 days post-infection).

Male Training Center for Family Planning & Reproductive Health (MTC), Office of Population Affairs, Department of Health and Human Services

In general, the MTC recommends at least annual testing for chlamydia, gonorrhea, syphilis, HIV/AIDS, and Hepatitis C for anyone in an at-risk population, including MSM. For syphilis, certain populations require testing at 3-6 month intervals, including those who exchange sex for drugs, commercial sex workers, and young MSM.

The MTC does not recommend screening for pharyngeal chlamydia infections. They do recommend follow-up test three months after initial positive chlamydia test. They recommend using a urine-based NAAT for chlamydia for at-risk male populations under the age of 25, which include MSM, patients at STI clinics, and military personnel (under the age of 30), and inmates entering jails or detention centers (under the age of 30). Men who have had receptive anal intercourse in the preceding year should have a NAAT performed on a rectal swab to check for rectal chlamydial infection.

The MTC recommends using NAAT for gonorrhea testing of at-risk male adolescents and adults, including MSM. "Males with gonorrhea infection should be re-screened for reinfection at 3 months." Annual exams for MSM include screening for urethral infections, pharyngeal infections using NAAT for those "who have had receptive oral intercourse" during the preceding year, and rectal infections using NAAT of rectal swabs for those "who have had receptive anal intercourse" during the preceding year. "More frequent STD screening (i.e., at 3 – 6 month intervals) is indicated for MSM who have multiple or anonymous partners" (Marcell & Health, 2014).

Canadian Guidelines on Sexually Transmitted Infections

"For anal warts, no specific testing is recommended to verify the presence or type of HPV as this will not alter management. Anal Pap and/or HPV testing may be of value to identify precancerous anal intraepithelial neoplasia (Marcell & Health) in high-risk groups… Although no products are currently licensed for these [pharyngeal] specimens in Canada, validated NAATs can be used to detect oropharyngeal *N. gonorrhoeae* and *C. trachomatis* infections. Confirmation of positives with culture or a second NAAT should be performed." NAAT can be performed on first-void urine samples from male patients or vaginal swabs or urine samples obtained from female patients. Since NAAT allows for the testing of antimicrobial susceptibility in gonorrheal infections, "depending on the clinical situation, consideration should be given to using both culture and NAAT, especially in symptomatic patients." For oral lesions of suspected HSV, they recommend using NAAT or to obtain fluid for culture. "NAATs approach sensitivities and specificities of 100%, with rapid turn-around of results." For syphilis, "NAATs can be used as a non-serological method for identifying *T. pallidum* in mucosa and skin involve. They are very sensitive and specific. When genital lesions characteristic of early syphilis are present, clear serous fluid may be collected for dark-field microscopy, enabling observation of morphology and movement of the spirochetes for the detection of *T. pallidum* (not reliable for oral or rectal lesions)" (Chernesky et al., 2017).

American Academy of Pediatrics (AAP)

Chlamydia: The AAP recommends annual screening for sexually active females 25 years old or younger. They also recommend annual urethral and rectal chlamydia screenings for sexually active MSM, but more frequent screening (every 3-6 months) for those who are in a higher risk category, such as multiple partners, sex-for-drugs, and so on. Anyone who has been exposed to chlamydia in the past 60 days should also be tested. "Consider screening sexually active males annually in settings with high prevalence rates, such as jails or juvenile corrections facilities, national job training programs, STD clinics, high school clinics, and adolescent clinics for patients who have a history of multiple partners." Anyone who has tested positive for chlamydia should be retested three months after receiving treatment.

Gonorrhea: Similar to chlamydia, the AAP recommends annual screening for sexually active females under the age of 25. "Routinely screen sexually active adolescent and young adults MSM for pharyngeal, rectal, and urethral gonorrhea infection annually if engaging in receptive oral or anal intercourse or insertive intercourse, respectively." Again, like chlamydial infections, those participating in higher risk activities should be tested every 3-6 months. Anyone who has been exposed to gonorrhea in the past 60 days should also be tested. Finally, the screening recommendations for other males are similar to the recommendations concerning chlamydial infections. Anyone who has tested positive for gonorrhea should be retested three months after receiving treatment.

Syphilis: "The routine screening of nonpregnant, heterosexual adolescents is not recommended. However, screening is recommended for all sexually active adolescent and young adults MSM annually or every 3 to 6 months if high risk and can be considered for youth whose behaviors put them at higher risk" (Murray et al., 2014).

National Institute for Health and Care Excellence (NICE)

NICE released their guidelines concerning cancer of the upper aerodigestive tract in 2016 (with updates in 2018 online). Recommendation 1.6.1: "Test all squamous cell carcinomas of the oropharynx using p16 immunohistochemistry. Regard the p16 test result as positive only if there is strong nuclear and cytoplasmic staining in more than 70% of tumour cells." In Recommendation 1.6.2: "Consider high-risk HPV DNA or RNA in-situ hybridisation in all p16-positive cancers of the oropharynx to confirm HPV status." In explaining their recommendations, NICE states, "HPV testing is currently recommended in cancer of the oropharynx because it has significant prognostic implication" (NICE, 2018).

Canadian Paediatric Society (CPS)

The 2024 update to the CPS practice point titled “Diagnosis and management of congenital syphilis – Avoiding missed opportunities" included the following:

> “The potential for asymptomatic syphilis infection and its nonspecific or subtle maternal disease manifestations make serology the cornerstone of diagnosis. At a minimum, syphilis serology is recommended at the time of the first prenatal visit, with recommendations for repeat testing at 28 to 32 weeks and at delivery in areas with outbreaks or for individuals with ongoing risk of infection. Repeat testing should also be performed in the context of clinical suspicion of maternal reinfection, a new maternal STI at any point during pregnancy (e.g., gonorrhea, chlamydia), in case of a stillbirth after 20 weeks gestation, or in accordance with provincial/territorial guidelines. Newborn infants ideally should not be discharged from hospital until results of maternal syphilis testing are known and appropriate steps for management are arranged. ” (Society, 2024).

The CPS practice point sexually transmitted infections in adolescents: Maximizing opportunities for optimal care (Allen et al., 2019) included the following table concerning what screening tests should be used for each condition. These guidelines were updated in 2019, and reaffirmed in 2020 (Allen et al., 2019).

Table 1: What screening tests should be used use to detect sexually transmitted infections?

What screening tests should be used use to detect sexually transmitted infections?		
Infection	**Screening tests/samples**	**Follow-up testing**
Chlamydia	NAAT is the most sensitive and specific test. Can be performed on urine, urethral swabs, vaginal or cervical swabs* A culture of cervical or urethral specimen is the test of choice for medico-legal cases (e.g., sexual assault). Confirmation by NAAT using a different set of primers or DNA sequencing may be used. For pharyngeal and rectal specimens, NAAT may be considered; discuss with testing laboratory	Test-of-cure 3 to 4 weeks after treatment: – Compliance is uncertain – Second-line or alternative treatment was used – Re-exposure risk is high – An adolescent is pregnant
Syphilis	Serology remains the usual diagnostic test unless the patient has lesions compatible with syphilis Treponemal-specific screening assays (e.g., EIA) are more sensitive than non-treponemal tests, though testing algorithms vary across jurisdictions If treponemal-specific assay is positive, a second treponemal test is usually required	Follow-up testing depends on the nature of infection, as follows: Primary, secondary, early latent infection: Repeat serology at 1, 3, 6, and 12 months after treatment Late latent infection: Repeat serology 12 and 24 months after treatment Neurosyphilis: Repeat 6, 12, and 24 months after treatment
Gonorrhea	NAAT can be used to detect gonorrhea from urine, and urethral, vaginal and cervical swabs in symptomatic and asymptomatic individuals*	Test-of-cure (culture 3 to 7 days post-treatment or NAAT 2 to 3 weeks later) if:

	Culture allows for antimicrobial susceptibility testing and should be performed if a patient does not promptly respond to therapy Cultures should be submitted for asymptomatic or symptomatic MSM, who have an increased incidence of antibiotic resistance For rectal and pharyngeal testing, discuss preferred specimens with the testing laboratory Culture is preferred for pharyngeal and rectal specimens For medico-legal purposes, a positive result obtained from NAATs should be confirmed using culture or a different set of primers, or by DNA sequencing techniques	– Second-line or alternative treatment was used – Antimicrobial resistance is a concern – Compliance is uncertain – Re-exposure risk is high – An adolescent is pregnant – Previous treatment failure – Pharyngeal or rectal infection – Infection is disseminated – Signs, symptoms persist post-treatment

**Discuss specimen selection to ensure that the NAAT is validated for the specimen to be collected and the patient being tested. For example, NAAT testing has not been validated for children ≤12 years of age and for medico-legal specimens.*

British Association for Sexual Health and HIV (BASHH)

UK National Guideline for the Management of Lymphogranuloma Venereum (White et al., 2013): "Commercial molecular diagnostic techniques to detect *C. trachomatis* remain the primary test of choice, with referral of *C. trachomatis*-positive specimens for molecular tests to confirm the presence of LGV-associated DNA." Testing should be performed on anyone exhibiting symptoms of an LGV infection, including hemorrhagic proctitis, primary lesions, suspected LGV-associated pharyngitis, secondary lesions, buboes, lymphadenitis, and/or lymphadenopathy. Main diagnostic techniques include using either NAATs, "culture on cycloheximide-treated McCoy cells of material from suspected LGV lesions," or serology testing. "Serology cannot necessarily distinguish past from current LGV infection, which might prove restrictive given the high number of recurrent LGV infections now seen in MSM."

UK National Guideline for the Management of Anogenital Herpes (Patel et al., 2015): The clinical signs and symptoms of an HSV infection can include "painful ulceration, dysuria, vaginal or urethral discharge" as well as systemic symptoms of fever and myalgia. Other signs can include bilateral lymphadenitis—although, alternating sides

can occur in subsequent episodes—and proctitis. With a Grade C recommendation, "The confirmation and typing of the infection and its type, by direct detection of HSV in genital lesions, are essential for diagnosis, prognosis, counselling, and management." BASHH gives an "A" recommendation of directly testing swabs from either anogenital lesions or the rectal mucosa in suspected proctitis. They recommend with a "B" rating that virus typing be performed to differentiate HSV-1 from HSV-2 in newly diagnosed cases of genital herpes. NAATs are the preferred testing method (grade "A" recommendation) since HSV culture tests can miss around 30% of PCR-positive samples.

UK National Guideline for the Management of Infection with Chlamydia Trachomatis (updated 2018) (Nwokolo et al., 2016): "Testing for genital and extra-genital chlamydia should be performed using NAATs (Grade B)." MSM who test positive for both HIV and chlamydia should be tested for LGV even if asymptomatic for the latter (Grade B). They give a Grade B recommendation for LGV testing in patients presenting with proctitis and a Grade C recommendation for treating both sexes presenting with proctitis the same.

The guidelines were updated in 2018, but NAAT testing is still considered the current standard of care for all chlamydia cases by the BASHH; "Although no test is 100% sensitive or specific, NAATs are known to be more sensitive and specific than EIAs" (BASHH, 2018).

UK National Guidelines on the Management of Syphilis (updated 2017, 2019) (Kingston et al., 2016): They recommend (2A) "where appropriate expertise and equipment are available, perform dark ground microscopy on possible chancres" and (1A) that "*T. pallidum* testing by PCR is appropriate on lesions where the organism may be expected to be located." Within the section on serology, they recommend (1B) that "An EIA/CLIA, preferably detecting both IgM and IgG is the screening test of choice"; "positive screening tests should be confirmed with a different treponemal test (not the FTA-abs) and a second specimen for confirmatory testing obtained" (1B); "a quantitative RPR or VDRL should be performed when screening tests are positive" (1A); and (1B) repeat testing for syphilis at 6 and 12 weeks if an isolated episode and "at two weeks after possible chancres that are dark-ground and/or PCR negative are observed." These guidelines were updated in 2017 and 2019, but diagnostic testing methods were not changed.

Infectious Diseases Working Party of the German Society for Hematology and Medical Oncology (AGIHO/DGHO) and the German Working Group for Blood and Marrow Transplantation (DAG-KBT)

In 2016, the AGIHO/DGHO and the DAG-KBT released the "Infectious diseases in allogeneic haematopoietic stem cell transplantation: prevention and prophylaxis strategy guidelines 2016". In this guideline, they note that "comprehensive pre-transplant assessment of the allogeneic haematopoietic stem cell transplantation (allo-HCT) recipient for infectious complications is a valuable tool to identify patients at increased risk for distinct infectious diseases. All candidates for allo-HCT should undergo a test for IgG antibodies specific for syphilis infection. Serologic testing for syphilis is recommended. Frequently TPHA/TPPA or VDRL are utilized. Important are the combinations of nontreponemal (e.g. VDRL) and treponemal tests. If a nontreponemal test is positive, confirmation of infection with treponemal test (e.g. TPPA or TP-EIA) should be performed" (Ullmann et al., 2016).

Cumulative Guideline Table

Year & Society	Condition	Microorganism	Recommendation
2023 NCCN	Anal Carcinoma	HPV	HPV linked to anal cancers and HPV positivity linked to positive OS
2024 NCCN	Cervical Cancer	HPV	Overwhelming evidence of link between HPV and cervical cancer; chronic HPV infection status used in aiding treatment/surgical options
2024 NCCN	Head and Neck Cancers/ Oropharyngeal Cancer	HPV	Requires HPV p16 testing by IHC; HPV status is imperative in determining therapy
2024 NCCN	Occult Primary Cancers (Squamous Cell Carcinoma)	HPV	If clinical presentation in the head and neck nodes is noted, check p16 IHC and ISH results
2023 NCCN	Penile Cancer	HPV	HPV linked to penile cancer; HPV status of lesions important for determining therapy
2024 NCCN	Vulvar Cancer (Squamous Cell Carcinoma)	HPV	HPV linked to vulvar cancer, especially HSIL; recommends HPV testing for "appropriate patients"

Year & Society	Condition	Microorganism	Recommendation
2021 USPSTF	NA	Chlamydia, Gonorrhea	Testing in sexually active women age 24 or younger and older women of at-risk populations; insufficient evidence concerning routinely screening in general population of males
2014 USPSTF	Oropharyngeal Cancer	HPV	Insufficient evidence to assess testing for HPV in cases of asymptomatic oropharyngeal cancer
2016 USPSTF	Asymptomatic Genital Herpes	HSV-2	Do not recommend testing asymptomatic patients for HSV-2
2016 USPSTF	NA	Syphilis	Grade A recommendation for screening asymptomatic patients of HIGH RISK categories but they do NOT recommend screening in asymptomatic patients not in high risk categories; recommend screening HIV-positive men and MSM every three months
2021 CDC	Genital, Anal, or Perianal Ulcers	Syphilis, HSV	Recommends syphilis serology, darkfield exam, or PCR testing if possible; culture or PCR for genital herpes; serologic testing for type-specific HSV antibody
2021 CDC	NA	Syphilis	Darkfield examination of exudate can be used for early diagnosis; presumptive diagnosis requires use of two tests—both a treponemal test and a non-treponemal test; any signs of CNS infection require additional testing
2021 CDC	NA	Chlamydia	Testing of women under age of 25 as well as older women and men if they fall in a high-risk category; do NOT recommend testing of asymptomatic men and older women

Year & Society	Condition	Microorganism	Recommendation
2021 CDC	NA	Gonorrhea	Testing of women under age of 25 as well as older women and men if they fall in a high-risk category; do NOT recommend testing of asymptomatic men and older women; men showing signs of urethral gonococcal infection should be tested
2021 CDC	NA	HPV	Recommends against using oncogenic HPV testing for asymptomatic men, women aged 25 and over, or for general STI testing. There is no approved test for HPV in men, and routine testing is not recommended for anal, penile, or throat cancers in men.
2021 CDC	Anogenital Warts	HPV	"HPV testing is not recommended for anogenital wart diagnosis, because test results are not confirmatory and do not guide genital wart management."
2021 CDC	Cervical Screening	HPV	For women aged 30 or older, HPV testing can be part of cervical screening. For women ages 30-65, if co-testing Pap test and HR-HPV, then frequency is every 5 years…if only doing a Pap test, the frequency is every 3 years HPV tests to screen for cervical cancer are recommended for women 30 years and older. They are not recommended to screen, men, adolescents, or women under the age of 30.

Year & Society	Condition	Microorganism	Recommendation
2019 IUSTI	Anogenital Warts	HPV	Do not recommend HPV testing for symptomatic anogenital warts since it adds no information for clinical use.
2020 IUSTI	NA	Gonorrhea	Culture testing is only method to determine antimicrobial susceptibility, but NAAT testing is more sensitive. Includes list of symptoms for testing.
2019 IUSTI	Lymphogranuloma venereum	Chlamydia	To diagnose LGV, a sample tested *C. trachomatis* positive with a commercial nucleic acid amplification test (NAAT) platform should be confirmed with an LGV discriminatory NAAT. For sensitive and specific LGV detection, laboratories are recommended to use a two-step procedure.
2014, 2020 IUSTI	NA	Syphilis	Like the CDC, they recommend a two-test method for diagnosing syphilis (one non-Treponema test and one Treponema test) if any initial screening test is positive
2015 IUSTI (published in 2016)	NA	Chlamydia	Recommends using an NAAT for chlamydia testing and lists signs/symptoms that require testing
2017 IUSTI	Genital herpes	HSV	Typically, does not recommend testing in asymptomatic patients; HSV DNA detection now replaces culture as gold standard
2014 MTC	NA	Chlamydia	Do not recommend pharyngeal screenings. Do recommend NAAT of at-risk groups with a 3-month follow-up test for patients who tested positive

Year & Society	Condition	Microorganism	Recommendation
2014 MTC	NA	Gonorrhea	Do recommend annual NAAT of at-risk groups with a 3-month follow-up test for patients who tested positive; more frequent testing in certain MSM populations
2014 MTC	NA	Syphilis	Do recommend annual testing of at-risk groups with 3-6 month testing of certain populations (commercial sex workers, inmates of correctional facilities, persons who exchange sex for drugs, and so on)
2017 Canadian Guidelines on STIs	NA	Chlamydia, Syphilis, Gonorrhea, HSV, and HPV	NAATs are more specific and sensitive than culture testing when available. For gonorrheal infections, only culture can test for antimicrobial susceptibility in gonorrhea.
2014 AAP	Adolescents & young adults	Chlamydia, Gonorrhea	All sexually active young women (under the age of 25) and MSM should have annual screenings. For those at higher risk, they should be screened every 3-6 months. Anyone who tests positive should be retested 3 months after receiving treatment.
2014 AAP	Adolescents & young adults	Syphilis	Do NOT recommend routine screening except for sexually active young MSM.
2016 NICE	Oropharyngeal Cancers	HPV	Test all carcinomas of the oropharynx using p16 IHC; consider using high-risk HPV DNA/RNA in situ hybridization in all p16-positive cancers
2018 CPS	Pregnant women	Syphilis	Testing at first prenatal visit as well as 28-32 weeks; if not tested during pregnancy, child does not leave the hospital without being tested

Year & Society	Condition	Microorganism	Recommendation
2020 CPS	Adolescents/young adults	Chlamydia, Syphilis, Gonorrhea	See detailed testing and frequency in table within the guidelines above
2015 BASHH (published in 2016)	NA	Syphilis	Dark-field microscopy or PCR tests can be performed. For serology, EIA/CLIA is the screening test of choice (preferably where both IgM and IgG are detected). Positive tests must be followed by a quantitative RPR or VDRL.
2013 BASHH	Suspected LGV	Chlamydia	Testing should use either NAAT, culture testing, or serology; however, the latter cannot distinguish current from past infections.
2014 BASHH (published in 2015)	Anogenital herpes	HSV	NAAT is preferred over other forms of testing ("A" grade). Differentiation of virus type should be determined on new cases of genital herpes ("B" grade).
2015, 2018 BASHH	NA	Chlamydia	Test for chlamydia using NAATs. Both sexes presenting with proctitis should be treated the same with respect to LGV testing. HIV-positive men with chlamydia should also be tested for LGV, even if asymptomatic.
Abbreviations: CLIA = chemiluminescent assay; EIA = enzyme immunoassay; GC = gonococcal; HPV = human papillomavirus; HR-HPV = high risk or oncogenic HPV testing; HSIL = high-grade squamous intraepithelial lesions; HSV = herpes simplex virus; IHC = immunohistochemistry; LGV = lymphogranuloma venereum; MSM = men having sex with men; NA = not applicable; NAAT = nucleic acid amplification testing; OS = overall survival; RPR = rapid plasma reagin test; VDRL = Venereal Diseases Research Laboratory carbon antigen test			

Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state

coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

The FDA has approved many tests for HSV, chlamydia, gonorrhea, and syphilis. Some of these tests are discussed in the "Proprietary Testing" section of this policy. In addition to these tests, many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
82565	Creatinine; blood
82575	Creatinine; clearance
84702	Gonadotropin, chorionic (hCG); quantitative
84703	Gonadotropin, chorionic (hCG); qualitative
86592	Syphilis test, non-treponemal antibody; qualitative (eg, VDRL, RPR, ART)
86593	Syphilis test, non-treponemal antibody; quantitative
86631	Antibody; Chlamydia
86632	Antibody; Chlamydia, IGM
86694	Antibody; herpes simplex, non-specific type test
86695	Antibody; herpes simplex, type 1
86696	Antibody; herpes simplex, type 2
86701	Antibody; HIV-1
86702	Antibody; HIV-2
86703	Antibody; HIV-1 and HIV-2, single result
86704	Hepatitis B core antibody (HBcAb); total
86705	Hepatitis B core antibody (HBcAb); IgM antibody
86706	Hepatitis B surface antibody (HBsAb)
86780	Antibody; Treponema pallidum
86803	Hepatitis C antibody
86804	Hepatitis C antibody; confirmatory test (eg, immunoblot)
87081	Culture, presumptive, pathogenic organisms, screening only

CPT	Code Description
87110	Culture, Chlamydia, any source
87181	Susceptibility studies, antimicrobial agent; agar dilution method, per agent (eg, antibiotic gradient strip)
87340	Infectious agent antigen detection by immunoassay technique, (eg, enzyme immunoassay [EIA], enzyme-linked immunosorbent assay [ELISA], fluorescence immunoassay [FIA], immunochemiluminometric assay [IMCA]) qualitative or semiquantitative; hepatitis B surface antigen (HBsAg)
87490	Infectious agent detection by nucleic acid (DNA or RNA); Chlamydia trachomatis, direct probe technique
87491	Infectious agent detection by nucleic acid (DNA or RNA); Chlamydia trachomatis, amplified probe technique
87492	Infectious agent detection by nucleic acid (DNA or RNA); Chlamydia trachomatis, quantification
87528	Infectious agent detection by nucleic acid (DNA or RNA); Herpes simplex virus, direct probe technique
87529	Infectious agent detection by nucleic acid (DNA or RNA); Herpes simplex virus, amplified probe technique
87530	Infectious agent detection by nucleic acid (DNA or RNA); Herpes simplex virus, quantification
87563	Infectious agent detection by nucleic acid (DNA or RNA); Mycoplasma genitalium, amplified probe technique
87590	Infectious agent detection by nucleic acid (DNA or RNA); Neisseria gonorrhoeae, direct probe technique
87591	Infectious agent detection by nucleic acid (DNA or RNA); Neisseria gonorrhoeae, amplified probe technique
87592	Infectious agent detection by nucleic acid (DNA or RNA); Neisseria gonorrhoeae, quantification
87623	Infectious agent detection by nucleic acid (DNA or RNA); Human Papillomavirus (HPV), low-risk types (eg, 6, 11, 42, 43, 44)
87624	Infectious agent detection by nucleic acid (DNA or RNA); Human Papillomavirus (HPV), high-risk types (eg, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68)
87625	Infectious agent detection by nucleic acid (DNA or RNA); Human Papillomavirus (HPV), types 16 and 18 only, includes type 45, if performed
87660	Infectious agent detection by nucleic acid (DNA or RNA); Trichomonas vaginalis, direct probe technique
87661	Infectious agent detection by nucleic acid (DNA or RNA); Trichomonas vaginalis, amplified probe technique

CPT	Code Description
87797	Infectious agent detection by nucleic acid (DNA or RNA), not otherwise specified; direct probe technique, each organism
87798	Infectious agent detection by nucleic acid (DNA or RNA), not otherwise specified; amplified probe technique, each organism
87799	Infectious agent detection by nucleic acid (DNA or RNA), not otherwise specified; quantification, each organism
87808	Infectious agent antigen detection by immunoassay with direct optical (ie, visual) observation; Trichomonas vaginalis
88341	Immunohistochemistry or immunocytochemistry, per specimen; each additional single antibody stain procedure (list separately in addition to code for primary procedure)
88342	Immunohistochemistry or immunocytochemistry, per specimen; initial single antibody stain procedure
88344	Immunohistochemistry or immunocytochemistry, per specimen; each multiplex antibody stain procedure
0064U	Antibody, Treponema pallidum, total and rapid plasma reagin (RPR), immunoassay, qualitative Proprietary test: BioPlex 2200 Syphilis Total & RPR Assay Lab/Manufacturer: Bio-Rad Laboratories
0065U	Syphilis test, non-treponemal antibody, immunoassay, qualitative (RPR) Proprietary test: BioPlex 2200 RPR Assay Lab/Manufacturer: Bio-Rad Laboratories
0096U	Human papillomavirus (HPV), high-risk types (ie, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68), male urine Proprietary test: HPV, High-Risk, Male Urine Lab/Manufacturer: Molecular Testing Labs/Roche Cobas
0210U	Syphilis test, non-treponemal antibody, immunoassay, quantitative (RPR) Proprietary test: BioPlex 2200 RPR Assay - Quantitative Lab/Manufacturer: Bio-Rad Laboratories
0402U	Infectious agent (sexually transmitted infection), Chlamydia trachomatis, Neisseria gonorrhoeae, Trichomonas vaginalis, Mycoplasma genitalium, multiplex amplified probe technique, vaginal, endocervical, or male urine, each pathogen reported as detected or not detected Proprietary test: Abbott Alinity™ m STI Assay Lab/Manufacturer: Abbott Molecular, Inc
0455U	Infectious agents (sexually transmitted infection), Chlamydia trachomatis, Neisseria gonorrhoeae, and Trichomonas vaginalis, multiplex amplified probe technique, vaginal, endocervical, gynecological specimens,

CPT	Code Description
	oropharyngeal swabs, rectal swabs, female or male urine, each pathogen reported as detected or not detected Proprietary test: Abbott AlinityTM m STI Assay Lab/Manufacturer: Abbott Molecular, Inc
0463U	Oncology (cervix), mRNA gene expression profiling of 14 biomarkers (E6 and E7 of the highest-risk human papillomavirus [HPV] types 16, 18, 31, 33, 45, 52, 58), by real-time nucleic acid sequence-based amplification (NASBA), exo- or endocervical epithelial cells, algorithm reported as positive or negative for increased risk of cervical dysplasia or cancer for each biomarker Proprietary test: Proofer '7 HPV mRNA E6 and E7 Biomarker Test Lab/Manufacturer: Global Diagnostics Labs, LLC, PreTect AS, a Mel-Mont Medical, Inc
0483U	Infectious disease (Neisseria gonorrhoeae), sensitivity, ciprofloxacin resistance (gyrA S91F point mutation), oral, rectal, or vaginal swab, algorithm reported as probability of fluoroquinolone resistance Proprietary test: Ciprofloxacin Susceptibility of Neisseria gonorrhoeae Lab/Manufacturer: MedArbor Diagnostics, SpeeDx, Inc
0484U	Infectious disease (Mycoplasma genitalium), macrolide sensitivity (23S rRNA point mutation), oral, rectal, or vaginal swab, algorithm reported as probability of macrolide resistance Proprietary test: Macrolide Resistance of Mycoplasma genitalium Lab/Manufacturer: MedArbor Diagnostics, SpeeDx, Inc
0500T	Infectious agent detection by nucleic acid (DNA or RNA), Human Papillomavirus (HPV) for five or more separately reported high-risk HPV types (eg, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68) (ie, genotyping)
G0432	Infectious agent antibody detection by enzyme immunoassay (EIA) technique, HIV-1 and/or HIV-2, screening
G0433	Infectious agent antibody detection by enzyme-linked immunosorbent assay (ELISA) technique, HIV-1 and/or HIV-2, screening
G0435	Infectious agent antibody detection by rapid antibody test, HIV-1 and/or HIV-2, screening
G0472	Hepatitis C antibody screening, for individual at high risk and other covered indication(s)
G0475	Hiv antigen/antibody, combination assay, screening
G0499	Hepatitis b screening in non-pregnant, high risk individual includes hepatitis b surface antigen (HBSAG) followed by a neutralizing confirmatory test for initially reactive results, and antibodies to HBSAG (anti-HBs) and Hepatitis B core antigen (anti-HBc)
S3645	HIV-1 antibody testing of oral mucosal transudate

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

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Revision History

Revision Date	Summary of Changes
09/04/2024	Reviewed and Updated: Updated the background, guidelines and recommendations, and evidence-based scientific references. Literature review necessitated the following changes to coverage criteria: New CC23: "23) Nucleic acid testing to determine antimicrobial susceptibility in N. gonorrhoeae or macrolide resistance in M. genitalium DOES NOT MEET COVERAGE CRITERIA." Added CPT code 0483U, 0484U Removed CPT code 0167U (deleted code; effective date 10/1/2024)
06/19/2024	Off-cycle coding modification: Added CPT code 0455U, 0463U (effective date 07/01/2024) Removed CPT code 0353U (effective date 07/01/2024)
03/06/2024	Off-cycle coding modification: Removed CPT code 0354U (effective date 04/01/2024)

Diagnostic Testing of Influenza

Policy Number: AHS – G2119 – Diagnostic Testing of Influenza	Prior Policy Name and Number, as applicable: • G2119 – Rapid Flu Tests in the Outpatient Setting • Also included influenza coverage from prior M2097 – Identification of Microorganisms Using Nucleic Acid Probes
Initial Presentation Date: 09/18/2015 Revision Date: February 1, 2025	

Policy Description

Influenza is an acute respiratory illness caused by influenza A or B viruses resulting in upper and lower respiratory tract infection, fever, malaise, headache, and weakness. It mainly occurs in outbreaks and epidemics during the winter season, and is associated with increased morbidity and mortality in certain high-risk populations (Dolin, 2022b).

Rapid influenza diagnostic tests (RIDTs) refer to clinical laboratory improvement amendments (CLIA) waived immunoassays that can detect influenza viruses during the outpatient visit, giving results in a clinically relevant time period to inform treatment decisions (CDC, 2017). Besides RIDTs, influenza can be detected using polymerase chain reaction (PCR)-based assays as well as culture testing; however, the former is not often used in initial clinical management due to time constraints. Serologic testing is not used in outpatient settings for diagnosis (Dolin, 2022a).

Related Policies

Policy Number	Policy Title
AHS-G2149	Pathogen Panel Testing
AHS-G2174	Coronavirus Testing in the Outpatient Setting

Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) For symptomatic individuals (see Note 1) (when influenza activity has been documented in the community or geographic area), **one**, but **not both**, of the following **MEETS COVERAGE CRITERIA**:
   a) One single rapid flu test (**either** a point-of-contact rapid nucleic acid amplification test (NAAT) **or** a rapid antigen test).
   b) One single traditional NAAT.
2) Viral culture testing for influenza **DOES NOT MEET COVERAGE CRITERIA**.
3) For asymptomatic individuals, influenza testing (e.g., rapid antigen flu tests, rapid NAAT or RT-PCR tests, traditional RT-PCR tests, viral culture testing) **DOES NOT MEET COVERAGE CRITERIA**.
4) Serology testing for influenza **DOES NOT MEET COVERAGE CRITERIA**.

NOTES:

Note 1: Typical Influenza Signs and Symptoms (CDC, 2020a):

- Fever: A 100.4°F or higher temperature or feeling feverish/chills AND one or more:
  - Cough
  - Sore throat
  - Headaches and/or body aches
  - Difficulty breathing or shortness of breath
  - Fatigue
  - Runny or stuffy nose

Table of Terminology

Term	Definition
AAEM	American Academy of Emergency Medicine
AAP	American Academy of Paediatrics
ATS	American Thoracic Society
CDC	Centres for Disease Control and Prevention
CLIA	Clinical Laboratory Improvement Amendments
DFA/IFA	Direct or Indirect fluorescent antibody staining
DNA	Deoxyribonucleic acid
EIA	Enzyme immunoassay
ELISA	Enzyme-linked immunosorbent assay
FBC	Full blood counts
FDA	Food and Drug Administration
FIA	Fluorescence immunoassay

ICT	Immunochromatographic
IDSA	Infectious Diseases Society of America
IMCA	Immunochemiluminometric assay
MDCK	Madin-Darby Canine Kidney
NAAT	Nucleic acid amplification test
NIBSC	National Institute for Biological Standards and Control
NIH	National Institute of Health
NPS	Nasopharyngeal Swab
NPV	Negative predictive value
PCR	Polymerase chain reaction
POC	Point-of-care
PPV	Positive predictive value
RAD	Rapid antigen diagnostic
RIDTs	Rapid influenza diagnostic tests
RSV	Respiratory syncytial virus
RT-PCR	Reverse-transcriptase polymerase chain reaction

Scientific Background

The influenza virus causes seasonal epidemics that result in severe illnesses and death every year. Influenza characteristically begins with the abrupt onset of fever, headache, myalgia, and malaise (Dolin, 1976; Kilbourne & Loge, 1950; Loeb et al., 2012; Nicholson, 1992), accompanied by manifestations of respiratory tract illness, such as nonproductive cough, sore throat, and nasal discharge (Dolin, 2022b).

High titers of influenza virus are often present in respiratory secretions of infected persons. Influenza is transmitted primarily via respiratory droplets produced from sneezing and coughing (Brankston et al., 2007; Dolin, 2022b; Mubareka et al., 2009) which requires close contact with an infected individual. The typical incubation period for influenza is one to four days (average two days) (CDC, 2017; Cox & Subbarao, 1999). The serial interval among household contacts is three to four days (Cowling et al., 2010). When initiated promptly (within the first 24 to 30 hours), antiviral therapy can shorten the duration of influenza symptoms by approximately one-half to three days (Cooper et al., 2003; Dobson et al., 2015; Hayden et al., 1997; Heneghan et al., 2014; Jefferson et al., 2014; Nicholson et al., 2000; Zachary, 2023).

In certain circumstances, the diagnosis of influenza can be made clinically, such as during an outbreak. At other times, it is important to establish the diagnosis using laboratory testing. Viral diagnostic test options include rapid antigen tests, immunofluorescence assays, and reverse-transcriptase polymerase chain reaction (RT-PCR)-based testing (CDC, 2017). Among these, RT-PCR is the most sensitive and specific (Dolin, 2022a). Rapid influenza antigen tests are immunoassays that can identify influenza A and B viral nucleoprotein antigens in respiratory specimens (CDC, 2017) which yield qualitative results in approximately 15 minutes or less. However, they have much lower sensitivity (CDC, 2017; Harper et al., 2009; Hurt et al., 2007; Ikenaga et al., 2008). A recent meta-analysis found that the sensitivity of these immunoassays was 62.3 percent and the specificity was 98.2 percent (Chartrand et al., 2012). Furthermore, detectable viral shedding in respiratory secretions peaks at 24 to 48 hours of illness and then rapidly declines (Dolin, 2022a).

A decision analysis by Sintchenko et al. (2002) concluded that treatment based on rapid diagnostic testing results was appropriate first over empirical antiviral treatment, except during influenza epidemics. When the probability of a case being due to influenza reached 42 percent, the two strategies were equivalent. Further, a separate meta-analysis found that rapid diagnostic testing did not add to the overall cost-effectiveness of treatment if the probability of influenza was greater than 25 to 30 percent (Call et al., 2005; Dolin, 2022a).

Analytical Validity

Viral culture is a gold standard for influenza diagnosis, but it is very time-consuming with an average 7-day turnaround time; on the other hand, real-time RT-PCR and shell vial (SV) testing require only an average of 4 hours and 48 hours, respectively. A study by Lopez Roa et al. (2011) compared real-time RT-PCR and SV testing against conventional cell culture to detect pandemic influenza A H1N1. The sensitivity of real-time RT-PCR as compared to viral culture testing was 96.5%, and SV had a sensitivity of 73.3% and 65.1%, depending on the use of either A549 cells or Madin-Darby Canine Kidney (MDCK) cells, respectively. The authors conclude, "Real-time RT-PCR displayed high sensitivity and specificity for the detection of influenza A H1N1 in adult patients when compared with conventional techniques" (Lopez Roa et al., 2011).

Clinical Utility and Validity

In 2017, Yoon et al. (2017) investigated the use of saliva specimens for detecting influenza A and B using RIDTs. Both saliva and nasopharyngeal swab (NPS) samples were analyzed from 385 patients; each sample was assayed using four different RIDTs—the Sofia Influenza A+B Fluorescence Immunoassay, ichroma TRIAS Influenza A+B, SD Bioline Influenza Ag, and BinaxNOW Influenza A/B antigen kit—as well as real-time RT-PCR. Using real-time RT-PCR as a standard, 31.2% of the patients tested positive for influenza A and 7.5% for influenza B. All four RIDTS had "slightly higher" diagnostic sensitivity in NPS samples than saliva samples; however, both Sofia and ichroma "were significantly superior to those of the other conventional influenza RIDTs with both types of sample" (Yoon et al., 2017). The authors note that the sensitivity of diagnosis improves if both saliva and NPS testing is performed (from 10% to 13% and from 10.3% to 17.2% for A and B, respectively). The researchers conclude, "This study demonstrates that saliva is a useful specimen for influenza detection, and that the combination of saliva and NPS could improve the sensitivities of influenza RIDTs" (Yoon et al., 2017).

Ryu et al. (2016) investigated the efficacy of using instrument-based digital readout systems with RIDTs. In their 2016 paper, the authors included 314 NPS samples from patients with suspected influenza and tested each sample with the Sofia Influenza A+B Fluorescence Immunoassay and BD Veritor System Flu A+B, which use instrument-based digital readout systems, as well as the SD Bioline assay (a traditional immunochromatographic assay) and PCR, the standard. Relative to the RT-PCR standard, for influenza A, the sensitivities for the Sofia, BD Veritor, and SD Bioline assays were 74.2%, 73.0%, and 53.9%, respectively; likewise, for influenza B, the sensitivities, respectively, were 82.5%, 72.8%, and 71.0%. All RIDTS show 100% specificities for both subtypes A and B. The authors conclude, "Digital-based readout systems for the detection of the influenza virus can be applied for more sensitive diagnosis in clinical settings than conventional [RIDTs]" (Ryu et al., 2016). Similar research was performed in 2018 on NPS using RIDTs with digital readout systems—Sofia and Veritor as before along with BUDDI—as compared to standard RT-PCR and the SD Bioline immunochromatographic assay (n=218). The four RIDTs were also tested with diluted solutions from the National Institute for Biological Standards and Control (NIBSC) to probe lower detection limits for each testing method. Again, the digital-based assays exhibited higher sensitivity for influenza. "Sofia showed the highest sensitivity for influenza A and B

detection. BUDDI and Veritor showed higher detection sensitivity than a conventional RIDT for influenza A detection. Further study is needed to compare the test performance of RIDTs according to specific, prevalent influenza subtypes" (Ryu et al., 2018).

Another study compared the Alere iNAT, a rapid isothermal nucleic acid amplification assay, to the Sofia Influenza A+B and the BinaxNOW Influenza A&B immunochromatographic (ICT) assay. Using RT-PCR as the standard for 202 NPS samples, the "Alere iNAT detected 75% of those positive by RT-PCR, versus 33.3% and 25.0% for Sofia and BinaxNOW, respectively. The specificity of Alere iNAT was 100% for influenza A and 99% for influenza B" (Hazelton et al., 2015). BinaxNOW also had a sensitivity of only 69% for influenza as compared to RT-PCR in another study of 520 NPS from children under the age of 5 (Moesker et al., 2016).

Young et al. (2017) investigated the accuracy of using point-of-care (POC) nucleic acid amplification test (NAAT)-based assays on NPS as compared to the US Food and Drug Administration (FDA)-cleared in vitro PCR test, GenMark Dx Respiratory Viral Panel. Their study consisted of 87 NPS samples from adults. As compared to the RT-PCR gold standard, the cobas Liat Influenza A/B POC test had an overall sensitivity and specificity of 97.9% and 97.5%, respectively, whereas the Alere i Influenza A&B POC test's sensitivity was only 63.8% with a specificity of 97.5% (Young et al., 2017). Taken together, the authors conclude that "the cobas Influenza A/B assay demonstrated performance equivalent to laboratory-based PCR, and could replace rapid antigen tests" (Young et al., 2017). These results are corroborated by another study that measured the specificity of the cobas POC assay as 100% for influenza A/B with a sensitivity of 96% for influenza A and 100% for influenza B (Melchers et al., 2017). Further, a third study reported a 6.5% invalid rate (as defined by as a failure on a first-run assay) by the cobas POC assay; however, "the sensitivities and specificities for all assays [cobas, Xpert Xpress Flu/RSV, and Aries Flu A/B & RSV] were 96.0 to 100.0% and 99.3 to 100% for all three viruses [influenza A, influenza B, and respiratory syncytial virus]" (Ling et al., 2018).

Antoniol et al. (2018) aimed to evaluate the usage of rapid influenza diagnostic tests (RIDTs) in adults, particularly the OSOM® Ultra Flu A&B on viral strains of influenza A/B in the emergency department. The diagnostic evaluation of this test was compared against the Xpert® Flu PCR test. The PCR test had a sensitivity of 98.4%, specificity of 99.7%, positive predictive value (PPV) of 99.2% and a negative predictive value (NPV) of 99.5%, whereas the OSOM® Ultra Flu A&B RIDT had a sensitivity of 95.1%, specificity of 98.4%, positive predictive value of 95.1%, and negative predictive value of 98.4%. However, "there was no difference in test performance between influenza A and B virus nor between the influenza A subtypes," thereby solidifying the use of both the PCR and RIDT in diagnosing influenza strains in adult and elderly patients (Antoniol et al., 2018).

Lee et al. (2019) conducted a systematic review and meta-analysis on point-of-care tests (POCTs) for influenza in ambulatory care settings. After screening, seven randomized studies and six non-randomized studies from studies mostly from pediatric emergency departments were included. The researchers concluded that "in randomized trials, POCTs had no effect on admissions (RR 0.93, 95% CI 0.61-1.42, I2 = 34%), returning for care (RR 1.00 95% CI = 0.77-1.29, I2 = 7%), or antibiotic prescribing (RR 0.97, 95% CI 0.82-1.15, I2 = 70%), but increased prescribing of antivirals (RR 2.65, 95% CI 1.95-3.60; I2 = 0%). Further testing was reduced for full blood counts (FBC) (RR 0.80, 95% CI 0.69-0.92 I2 = 0%), blood cultures (RR 0.82, 95% CI 0.68-0.99; I2 = 0%) and chest radiography (RR 0.81, 95% CI 0.68-0.96; I2 = 32%), but not urinalysis (RR 0.91, 95% CI 0.78-w1.07; I2 = 20%)." Among the non-randomized studies, fewer reported these outcomes, with some showing inconsistency with the randomized trial outcomes, such as there being fewer antibiotic prescriptions and less urinalysis testing. This demonstrated the use of POCTs for influenza and how they influence clinical treatment and decision making (Lee et al., 2019).

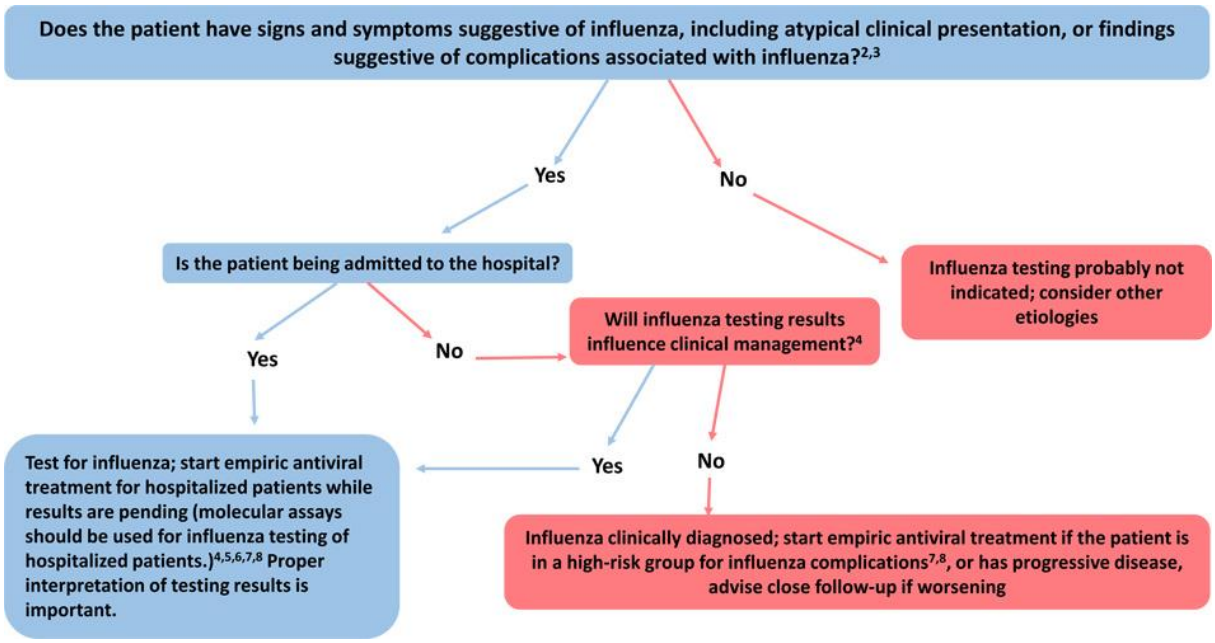
Kanwar et al. (2020) compared three rapid, POC molecular assays for influenza A and B detection in children: the ID Now influenza A & B assay, the Cobas influenza A/B NAAT, and Xpert Xpress Flu. Each of the three aforementioned tests are CLIA-waived influenza assays. PCR was used to compare results from each. NPS Samples from 201 children were analyzed for this study. The researchers note that "The overall sensitivities for the ID Now assay, LIAT, and Xpert assay for Flu A virus detection (93.2%, 100%, and 100%, respectively) and Flu B virus detection (97.2%, 94.4%, and 91.7%, respectively) were comparable. The specificity for Flu A and B virus detection by all methods was >97%" (Kanwar et al., 2020).

Sato et al. (2022) conducted a study comparing the results from rapid antigen detection (Quick Chaser Flu A, B), silver amplified immunochromatography (Quick Chaser Auto Flu A, B), and two separate NAATs (Xpert Xpress Flu/RSV and cobas Influenza A/B & RSV). The researchers also used a baseline RT-PCR assay as a reference for the study results. The sensitivities of the rapid antigen detection test and silver amplified immunochromatography test were 41.7% and 50.0% <6 hours after onset, but both were 100% in sensitivity at 24-48h after onset. Ultimately, the researchers concluded that the two NAATs had comparable analytical performances, whereas the rapid antigen detection and silver amplified immunochromatography tests had increased false negatives oftentimes when viral load is low in early infection (Sato et al., 2022).

Guidelines and Recommendations

Centers for Disease Control and Prevention (CDC)

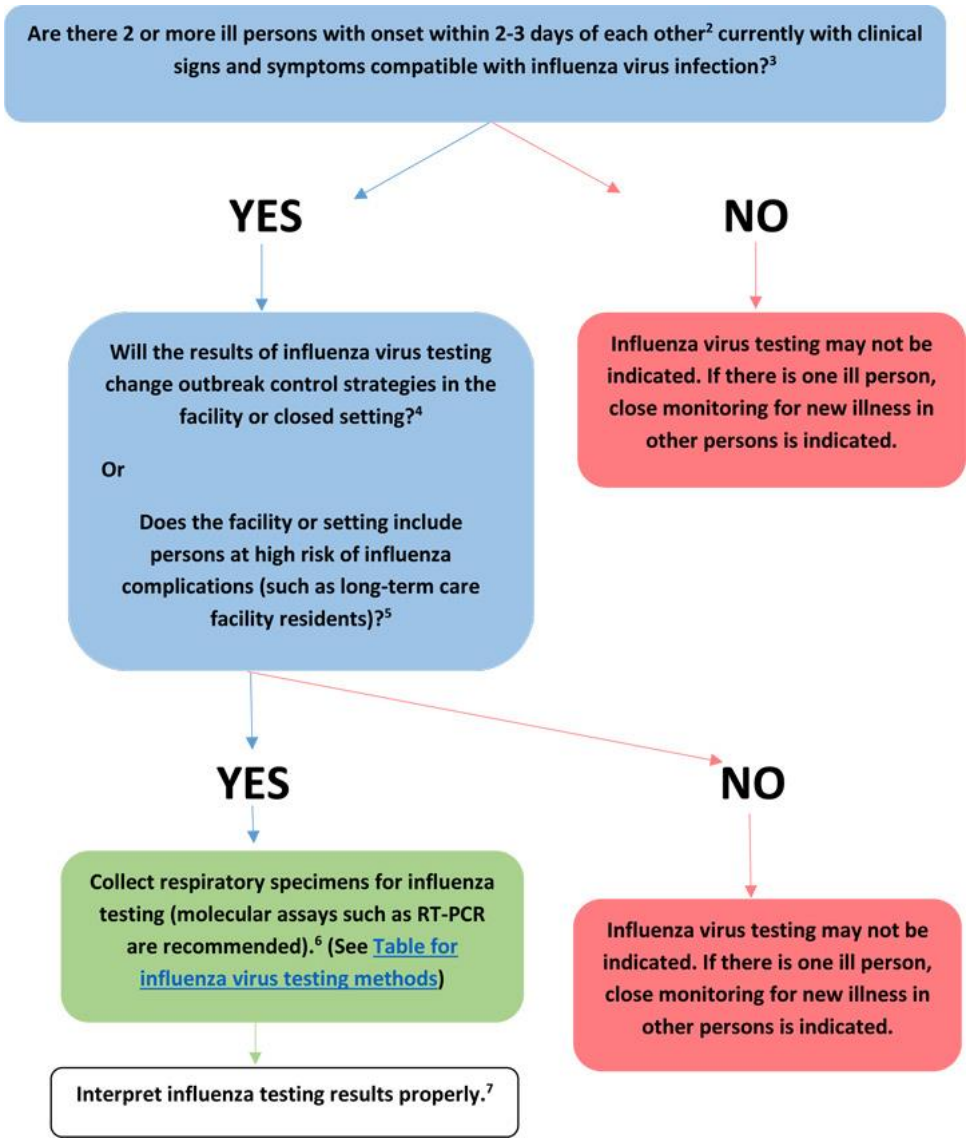
The CDC gives two sets of guidelines concerning testing for influenza. If influenza is known to be circulating in the community, they give the algorithm displayed in the figure below (CDC, 2020b):



If the patient is asymptomatic for influenza, then they do not recommend testing. If the patient is symptomatic and is being admitted to the hospital, then they recommend testing; on the other hand, if a symptomatic patient is not being admitted to the hospital, they recommend testing if the results of the test will influence clinical management. Otherwise, if the test results are not going to influence the

clinical management, then do not test but do administer empiric antiviral treatment for any patient in high-risk categories (CDC, 2020b). [For a list of typical signs and symptoms of influenza according to the CDC, please refer to Note 1 within the Coverage criteria section above (CDC, 2020a).]

For possible outbreaks in a closed setting or institution, the CDC issued the guideline algorithm in the figure below (CDC, 2019):



If only one person is showing signs and symptoms of influenza, then testing is not recommended but he/she should be closely monitored. If multiple people are showing signs of influenza, then RT-PCR testing is recommended if the results would change control strategies or if there are persons at high risk of complications within the facility or closed setting (CDC, 2019). [For a list of signs and symptoms and a list of high-risk populations, please see Notes 1 and 2, respectively, in the Coverage criteria section above] (CDC, 2020a).

The CDC notes the usefulness of RIDT influenza testing given the rapid testing time (less than 15 minutes on the average) and that some have been cleared for point-of-care use, but they note the

limited sensitivity to detect influenza as compared to the reference standards for laboratory confirmation testing, RT-PCR or viral culture. Disadvantages of RIDTs include high false negative results, especially during outbreaks, false positive results during times when influenza activity is low, and the lack of parity in RIDTs in detecting viral antigens. "Testing is not needed for all patients with signs and symptoms of influenza to make antiviral treatment decisions…Once influenza activity has been documented in the community or geographic area, a clinical diagnosis of influenza can be made for outpatients with signs and symptoms consistent with suspected influenza, especially during periods of peak influenza activity in the community" (CDC, 2017).

The CDC notes the practicality of using RIDTs to detect possible influenza outbreaks, especially in closed settings. "RIDTs can be useful to identify influenza virus infection as a cause of respiratory outbreaks in any setting, but especially in institutions (i.e., nursing homes, chronic care facilities, and hospitals), cruise ships, summer camps, schools, etc. Positive RIDT results from one or more ill persons with suspected influenza can support decisions to promptly implement infection prevention and control measures for influenza outbreaks. However, negative RIDT results do not exclude influenza virus infection as a cause of a respiratory outbreak because of the limited sensitivity of these tests. Testing respiratory specimens from several persons with suspected influenza will increase the likelihood of detecting influenza virus infection if influenza virus is the cause of the outbreak, and use of molecular assays such as RT-PCR is recommended if the cause of the outbreak is not determined and influenza is suspected. Public health authorities should be notified promptly of any suspected institutional outbreak and respiratory specimens should be collected from ill persons (whether positive or negative by RIDT) and sent to a public health laboratory for more accurate influenza testing by molecular assays and viral culture." The CDC recommends using a molecular assay, such as RT-PCR, to test any hospitalized individual with suspected influenza rather than using an RIDT (CDC, 2017).

Infectious Diseases Society of America (IDSA)

The IDSA published an update to seasonal influenza in adults and children in 2018. Here, IDSA propounded the following patient populations as targets for influenza testing:

"Outpatients (Including Emergency Department Patients)

1. During influenza activity (defined as the circulation of seasonal influenza A and B viruses among persons in the local community) . . .:
    - Clinicians should test for influenza in high-risk patients, including immunocompromised persons who present with influenza-like illness, pneumonia, or nonspecific respiratory illness (eg, cough without fever) if the testing result will influence clinical management *(A–III)*.
    - Clinicians should test for influenza in patients who present with acute onset of respiratory symptoms with or without fever, and either exacerbation of chronic medical conditions (eg, asthma, chronic obstructive pulmonary disease [COPD], heart failure) or known complications of influenza (eg, pneumonia) if the testing result will influence clinical management *(A-III)* . . .
    - Clinicians can consider influenza testing for patients not at high risk for influenza complications who present with influenza-like illness, pneumonia, or nonspecific respiratory illness (eg, cough without fever) and who are likely to be discharged home if the results might influence antiviral treatment decisions or reduce use of unnecessary antibiotics, further diagnostic testing, and time in the emergency department, or if the results might influence antiviral treatment or chemoprophylaxis decisions for high-risk household contacts . . . *(C-III)*.
2. During low influenza activity without any link to an influenza outbreak:

- Clinicians can consider influenza testing in patients with acute onset of respiratory symptoms with or without fever, especially for immunocompromised and high-risk patients *(B-III)*.

Hospitalized Patients

3. During influenza activity:
   - Clinicians should test for influenza on admission in all patients requiring hospitalization with acute respiratory illness, including pneumonia, with or without fever *(A-II)*.
   - Clinicians should test for influenza on admission in all patients with acute worsening of chronic cardiopulmonary disease (eg, COPD, asthma, coronary artery disease, or heart failure), as influenza can be associated with exacerbation of underlying conditions *(A-III)*.
   - Clinicians should test for influenza on admission in all patients who are immunocompromised or at high risk of complications and present with acute onset of respiratory symptoms with or without fever, as the manifestations of influenza in such patients are frequently less characteristic than in immunocompetent individuals *(A-III)*.
   - Clinicians should test for influenza in all patients who, while hospitalized, develop acute onset of respiratory symptoms, with or without fever, or respiratory distress, without a clear alternative diagnosis *(A-III)*.
4. During periods of low influenza activity:
   - Clinicians should test for influenza on admission in all patients requiring hospitalization with acute respiratory illness, with or without fever, who have an epidemiological link to a person diagnosed with influenza, an influenza outbreak or outbreak of acute febrile respiratory illness of uncertain cause, or who recently traveled from an area with known influenza activity *(A-II)*.
   - Clinicians can consider testing for influenza in patients with acute, febrile respiratory tract illness, especially children and adults who are immunocompromised or at high risk of complications, or if the results might influence antiviral treatment or chemoprophylaxis decisions for high-risk household contacts . . . *(B-III)*" (Uyeki et al., 2018).

The following three recommendations relating to the type of outpatient influenza testing were published also included:

- "Clinicians should use rapid molecular assays (ie, nucleic acid amplification tests) over rapid influenza diagnostic tests (RIDTs) in outpatients to improve detection of influenza virus infection."
- "Clinicians should not use viral culture for initial or primary diagnosis of influenza because results will not be available in a timely manner to inform clinical management (A-III), but viral culture can be considered to confirm negative test results from RIDTs and immunofluorescence assays, such as during an institutional outbreak, and to provide isolates for further characterization."
- "Clinicians should not use serologic testing for diagnosis of influenza because results from a single serum specimen cannot be reliably interpreted, and collection of paired (acute/convalescent) sera 2–3 weeks apart are needed for serological testing" (Uyeki et al., 2018).

The 2018 IDSA guidelines for the diagnosis of infectious diseases by microbiology laboratories (Miller et al., 2018) under viral pneumonia respiratory infections, specifically including influenza, state: "Rapid antigen tests for respiratory virus detection lack sensitivity and depending upon the product, specificity. A recent meta-analysis of rapid influenza antigen tests showed a pooled sensitivity of 62.3% and a

pooled specificity of 98.2%. They should be considered as screening tests only. At a minimum, a negative result should be verified by another method… Several US Food and Drug Administration (FDA)-cleared NAAT platforms are currently available and vary in their approved specimen requirements and range of analytes detected" (Miller et al., 2018). Moreover, they state that the "IDSA/American Thoracic Society (ATS) practice guidelines (currently under revision) consider diagnostic testing as optional for the patient who is not hospitalized." For children, though, they do recommend testing for viral pathogens in both outpatient and inpatient settings. In the section on general influenza virus infection, again they recommend the use of rapid testing assays, noting the higher sensitivity of the NAAT-based methods over the rapid antigen detection assays. They also state: Serologic testing is not useful for the routine diagnosis of influenza due to high rates of vaccination and/or prior exposure" (Miller et al., 2018).

American Academy of Emergency Medicine (AAEM)

The AAEM approved a clinical practice paper on influenza in the emergency department: vaccination, diagnosis, and treatment. This document provides a "Level B" recommendation, stating "Testing for influenza should only be performed if the results will change clinical management. If a RAD [rapid antigen diagnostic] testing method is utilized, the provider should be aware of the limited sensitivity and the potential for false negatives. If clinical suspicion is moderate to high and RAD test is negative, one should consider sending a confirmatory RT-PCR or proceeding with empiric treatment for suspected influenza" (Abraham et al., 2016). This guideline has since been archived on the AAEM website.

Committee on Infectious Diseases, American Academy of Pediatrics (AAP), 32nd Edition (2021-2024, Red Book)

The Committee on Infectious Diseases released joint guidelines with the American Academy of Pediatrics. These joint guidelines recommend that "influenza testing should be performed when the results are anticipated to influence clinical management (eg, to inform the decision to initiate antiviral therapy or antibiotic agents, to pursue other diagnostic testing or to implement infection prevention and control measures)" (AAP, 2021).

Regarding types of testing, the AAP states that "The decision to test is related to the level local influenza activity, clinical suspicion for influenza, and the sensitivity and specificity of commercially available influenza tests… These include rapid molecular assays for influenza RNA or nucleic acid detection, reverse transcriptase-polymerase chain reaction (RT-PCR) single-plex or multiplex assays, real time or other RNA-based assays, immunofluorescence assays (direct [DFA] or indirect [IFA] fluorescent antibody staining) for antigen detection, rapid influenza diagnostic tests (RIDTs) based on antigen detection, rapid cell culture (shell vial culture), and viral tissue cell culture (conventional) for virus isolation. The optimal choice of influenza test depends on the clinical setting" (AAP, 2021).

National Institute of Health (NIH)

The NIH published a webpage on influenza diagnoses. This page notes that "Diagnostics that enable healthcare professionals to quickly distinguish one flu strain from another at the point of patient care and to detect resistance to antiviral drugs would ensure that patients receive the most appropriate care" (NIH, 2017).

Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

On January 12, 2017, the FDA released the following concerning the reclassification of influenza testing systems: "The Food and Drug Administration (FDA) is reclassifying antigen based rapid influenza virus antigen detection test systems intended to detect influenza virus directly from clinical specimens that are currently regulated as influenza virus serological reagents from class I into class II with special controls and into a new device classification regulation" (Kux, 2017). The effective date is February 13, 2017. This reclassification now requires new minimum standards and annual reactivity testing. "Consequently, many previously available RIDTs can no longer be purchased in the United States" (Azar & Landry, 2018).

A list of tests granted waived status under CLIA (Clinical Laboratory Improvement Amendments of 1988) according to CPT codes is maintained by the Centers for Medicare & Medicaid Services (CMS) website (CMS, 2018). As of August 14, 2023, 27 different influenza tests are listed with the 87804 CPT code for influenza immunoassay with direct optical observation.

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
87804	Infectious agent antigen detection by immunoassay with direct optical (ie, visual) observation; Influenza
87400	Infectious agent antigen detection by immunoassay technique, (eg, enzyme immunoassay [EIA], enzyme-linked immunosorbent assay [ELISA], fluorescence immunoassay [FIA], immunochemiluminometric assay [IMCA]) qualitative or semiquantitative; Influenza, A or B, each
87501	Infectious agent detection by nucleic acid (DNA or RNA); influenza virus, includes reverse transcription, when performed, and amplified probe technique, each type or subtype
87502	Infectious agent detection by nucleic acid (DNA or RNA); influenza virus, for multiple types or sub-types, includes multiplex reverse transcription, when performed, and multiplex amplified probe technique, first 2 types or sub-types

87503	Infectious agent detection by nucleic acid (DNA or RNA); influenza virus, for multiple types or sub-types, includes multiplex reverse transcription, when performed, and multiplex amplified probe technique, each additional influenza virus type or sub-type beyond 2 (List separately in addition to code for primary procedure)
86710	Antibody; influenza virus
87275	Infectious agent antigen detection by immunofluorescent technique; influenza B virus
87276	Infectious agent antigen detection by immunofluorescent technique; influenza A virus
87631	Infectious agent detection by nucleic acid (DNA or RNA); respiratory virus (eg, adenovirus, influenza virus, coronavirus, metapneumovirus, parainfluenza virus, respiratory syncytial virus, rhinovirus), includes multiplex reverse transcription, when performed, and multiplex amplified probe technique, multiple types or subtypes, 3-5 targets
87254	Virus isolation; centrifuge enhanced (shell vial) technique, includes identification with immunofluorescence stain, each virus

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

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Revision History

Revision Date	Summary of Changes
09/06/2023	Reviewed and Updated: Updated the background, guidelines and recommendations, and evidence-based scientific references. Literature review did not necessitate any modifications to coverage criteria. The following edits were made for clarity: All CC edited for clarity and consistency

Diagnostic Testing of Iron Homeostasis & Metabolism

Policy Number: AHS – G2011 – Diagnostic Testing of Iron Homeostasis and Metabolism	Initial Presentation Date: 11/16/2015 Effective Date: 4/1/2025

Policy Description

Iron, an essential nutrient with a variety of biological uses, is tightly regulated *in vivo* to maintain homeostasis. Enterocytes absorb iron as Fe^{2+} either in its non-heme form via DMT1 (divalent metal-ion transporter-1) or in heme form presumably through receptor-mediated endocytosis. The enterocytes then release iron through ferroportin where transferrin binds it as biologically inactive Fe^{3+}. Saturated transferrin delivers iron to erythrocyte precursors in bone marrow where it is incorporated into hemoglobin during erythropoiesis. Transferrin may also salvage iron released by the reticuloendothelial system and macrophages (Knutson, 2017).

All cells require iron; consequently, saturated transferrin can also bind to its receptors (TfR1 or TfR2). The bound transferrin receptor (TfR) undergoes receptor-mediated endocytosis followed by export of divalent iron for cellular use (Byrne et al., 2013). Intracellularly, iron is stored within the central cavity of the protein ferritin, a large spherical protein that can store up to 4500 iron atoms per protein. Ferritin has ferroxidase activity required for iron uptake and storage. In conjunction with transferrin and TfR, ferritin is an acute phase reactant that responds to oxidative stress and inflammation (Camaschella & Weiss, 2024). Moreover, TfR1 and TfR2, upon activation by transferrin, can initiate signaling cascades required for hepcidin expression (Roetto et al., 2018). Hepcidin, a small peptide hormone, acts as a

modulator of serum iron concentrations by binding to ferroportin, the only iron exporter; ultimately, this results in the degradation of ferroportin and an intracellular accumulation of iron (Pietrangelo, 2015).

Terms such as male and female are used when necessary to refer to sex assigned at birth. Please note that carbohydrate-deficient transferrin is out of scope for this policy.

Related Policies

Policy Number	Policy Title
AHS-M2012	Genetic Testing for Hereditary Hemochromatosis

Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) Measurement of serum ferritin levels **MEETS COVERAGE CRITERIA** in **any** of the following situations:
    a) For the evaluation of an individual with abnormal hemoglobin and/or hematocrit levels.
    b) For the evaluation and monitoring of iron overload disorders.
    c) For individuals with symptoms of hemochromatosis (see Note 1).
    d) For individuals with first-degree relatives (see Note 2) with confirmed hereditary hemochromatosis (HH).
    e) For the evaluation of individuals with liver disease.
    f) For the evaluation of hemophagocytic lymphohistiocytosis (HLH) and Still Disease.
    g) In males with secondary hypogonadism.
    h) At a frequency of every 1 to 3 months:
        i) For the evaluation and monitoring of patients with chronic kidney disease who are receiving or being considered for receiving treatment for anemia.
        ii) For individuals on iron therapy.
2) Measurement of serum transferrin saturation **MEETS COVERAGE CRITERIA** in **any** of the following:
    a) For the evaluation of iron overload in individuals with symptoms of hemochromatosis (see Note 1).
    b) For the evaluation of iron overload in individuals with first-degree relatives (see Note 2) with confirmed hereditary hemochromatosis (HH).
    c) For the evaluation of iron deficiency anemia.
3) For all other situations not addressed above, measurement of ferritin or transferrin levels, including transferrin saturation, **DOES NOT MEET COVERAGE CRITERIA**.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

4) Serum hepcidin testing, including immunoassays, **DOES NOT MEET COVERAGE CRITERIA**.

5) The use of GlycA testing to measure or monitor transferrin or other glycosylated proteins **DOES NOT MEET COVERAGE CRITERIA**.

NOTES:

Note 1: Symptoms of hemochromatosis, according to the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) of the National Institutes of Health include the following (NIDDK, 2020):

- Joint pain
- Fatigue
- Unexplained weight loss
- Abnormal bronze or gray skin color
- Abdominal pain
- Loss of sex drive

Note 2: First-degree relatives include parents, full siblings, and children of the individual.

Table of Terminology

Term	Definition
25(OH) vitamin D	25-hydroxy-vitamin D
AAFP	American Academy of Family Physicians
ACG	American College of Gastroenterology
AGA	American Gastroenterological Association
ASCO	American Society of Clinical Oncology
ASH	American Society of Hematology
BMP-SMAD	Bone morphogenetic protein-Smad
BPAN	Beta-propeller protein-associated neurodegeneration
BRINDA	Biomarkers reflecting the inflammation and nutritional determinants of anemia
▪ TM	Beta thalassemia major
CBC	Complete blood cell count
CHF	Congestive heart failure
CKD	Chronic kidney disease
CLIA ’88	Clinical Laboratory Improvement Amendments of 1988
CLSI-C62A	Clinical and Laboratory Standards Institute-C62A
CMS	Centers for Medicare and Medicaid Services
CRP	C-reactive protein
CVs	Coefficients-of-variation
DMT1	Divalent metal-ion transporter-1
DUOX2	*Dual oxidase 2*
ECCO	European Crohn’s and Colitis Organisation
ELISA	Enzyme-linked immunosorbent assay

ESAs	Erythropoiesis-stimulating agents
F5	*Coagulation factor V*
Fe2+	Ferrous ion
Fe3+	Ferric ion
FBC	Full blood count
FDA	Food and Drug Administration
FTH	Ferritin H
FTL	Ferritin L
FTL1	Ferritin light polypeptide 1
GDF-15	Growth differentiation factor 15
GlycA	Glycoprotein acetylation
GPX4	Glutathione peroxidase 4
GRE	Gradient recalled echo
GSH	Glutathione
HAMP	*Hepcidin antimicrobial peptide*
HEIRS	Hemochromatosis and iron overload screening
HFE	*Homeostatic iron regulator*
HH	Hereditary hemochromatosis
HLH	Hemophagocytic lymphohistiocytosis
HPLC/MS/MS	High-performance liquid chromatography/tandem mass spectrometry
hsCRP	High-sensitivity C-reactive protein
IBD	Inflammatory bowel disease
ICCAMS	International Consensus Conference on Anemia Management in Surgical Patients
ID	Iron deficiency
IDA	Iron deficiency anemia
IL-6	Interleukin-6
IRP	Iron responsive proteins
ISN	International Society of Nephrology
KDIGO	Kidney Disease: Improving Global Outcomes
LC-MS/MS	Light chromatography with tandem mass spectroscopy
LDTs	Laboratory-developed tests
LPI	Labile plasma iron
MCV	Mean corpuscular volume
MDS	Myelodysplastic syndrome
MPAN	Mitochondrial membrane protein-associated neurodegeneration
MRI	Magnetic resonance imaging
NBIA	Neurodegeneration with brain iron accumulation
NCOA4	Nuclear receptor coactivator 4
NF	Neuroferritinopathy
NICU	Neonatal intensive care unit
NIDDK	National Institute of Diabetes and Digestive and Kidney Diseases
NKF-KDOQI	The National Kidney Foundation-Kidney Disease Outcomes Quality Initiative
NMR	Nuclear magnetic resonance

NTBI	Non-transferrin-bound iron
PKAN	Pantothenate kinase-associated neurodegeneration
RBC	Red blood cell
RET-He	Reticulocyte hemoglobin equivalent
RDW	Red cell distribution width
ROS	Reactive oxygen species
SCD	Sickle cell disease
SF	Serum ferritin
SLC11A2	*Solute carrier family 11 member 2*
SLE	Systemic lupus erythematosus
SOFA	Severity of organ failure
SWI	Susceptibility weighted imaging
TfR	Transferrin receptor
TfR1	Transferrin receptor 1
TfR2	Transferrin receptor 2
TfS/TSAT	Transferrin saturation
TMPRSS6	*Transmembrane protease, serine 6*
USPSTF	United States Preventive Services Task Force
WHO	World Health Organization

Scientific Background

Iron is necessary for fundamental metabolic processes and acts as the central component in the catalytic sites of numerous essential enzymes and multiprotein complexes, such as mitochondrial respiratory chain complexes and oxygen binding proteins (Hentze et al., 2004; Zhang et al., 2014). Tight regulation of iron metabolism for maintaining adequate iron levels is achieved by the interaction of a number of iron metabolism-related proteins (Zhang et al., 2014) as well as the hemostatic modulation of iron absorption, utilization, and recycling (Hentze et al., 2010). This strict regulation is pertinent due to the potential toxicity of iron from its redox reactivity and the resultant generation of damaging free radicals (Finazzi & Arosio, 2014).

Several mechanisms in the body regulate the dietary absorption of iron and its concentration in other areas, such as plasma and extracellular milieu; this process is known as systemic iron homeostasis (Ganz, 2013). Iron homeostasis is a complex process where the small peptide hormone hepcidin plays a major role by binding the sole mammalian iron exporter, ferroportin. This leads to ferroportin degradation by lysosomes. Furthermore, hepcidin production is sensitive to extracellular iron concentrations by way of the human homeostatic iron regulator (HFE) protein and the transferrin receptors (TfRs). The HFE protein has been shown to interact with both TfR1 and TfR2, initiating the BMP-SMAD signaling pathway upon transferrin binding. This signaling cascade ultimately increases expression of the *HAMP* gene that encodes for hepcidin (Pietrangelo, 2015; Vujić, 2014).

Ferritins are a highly conserved family of proteins that detoxify and store excess iron as less reactive ferrihydrite (Hentze et al., 2004). This intracellular iron storage mechanism allows the cell to maintain and utilize spare iron based on changes in metabolic demand (Finazzi & Arosio, 2014). Mammalian ferritins are heteropolymers comprised of tissue-specific combinations of 24 subunits. These subunits consist of two types: Ferritin L (FTL) and Ferritin H (FTH); a spherical structure is formed from these

subunits, facilitating the dynamic storage of iron (Finazzi & Arosio, 2014; Liu & Theil, 2005). The levels and composition of ferritin are regulated by oxidative stress at the transcriptional level (Arosio & Levi, 2010; Bresgen & Eckl, 2015), and by iron responsive proteins (IRP) at the post-transcriptional level (Anderson et al., 2012). Several tissues express a mitochondria-specific ferritin protein that further protect these mitochondria from oxidative damage (Campanella et al., 2009; Paul et al., 2017).

Iron is released as needed from ferritin by ferritinophagy, the targeting of ferritin for degradation by lysosomes; this process requires cargo protein nuclear receptor coactivator 4 (NCOA4), as NCOA4-deficient cells cannot degrade ferritin correctly (Mancias et al., 2014). After release, the iron is transported back to the cytosol by divalent metal transporter 1 (DMT1) (La et al., 2018). This process allows the iron to become available as part of the labile iron pool (Cabantchik, 2014; Kruszewski, 2003).

Degradation of ferritin and resultant accumulation of lethal reactive oxygen species (ROS) has been recognized as a distinct iron-dependent type of regulated, non-apoptotic cell death known as ferroptosis (Hou et al., 2016; Xie et al., 2016). Dysregulated ferroptosis has been implicated in neurotoxicity, neurodegenerative diseases, acute renal failure, drug-induced hepatotoxicity, hepatic and heart ischemia/reperfusion injury, and T-cell immunity (Xie et al., 2016). Abnormal ferroptosis has also been recently found to play a role in drug treatment, particularly in decitabine treatment of myelodysplastic syndrome (MDS). The drug-induced ROS release decreases glutathione (GSH) and glutathione peroxidase 4 (GPX4), features characteristic of this unique cell-death process (Lv et al., 2020).

Ferritin can routinely be detected in serum (Alfrey, 1978) as a result of secretion from macrophages (Cohen et al., 2010) or release during cell death and lysis (Kell & Pretorius, 2014). Serum ferritin (SF) is primarily composed of L subunits, contains relatively little iron, and is partially glycosylated (Santambrogio et al., 1987; Wang et al., 2010). Causes of elevated SF levels include, but are not limited to, acute or chronic inflammation, chronic alcohol consumption, liver disease, renal failure, metabolic syndrome, or malignancy rather than iron overload (Koperdanova & Cullis, 2015). In healthy adults, levels of SF generally reflect overall iron storage (Costa Matos et al., 2013; Enko et al., 2015; Finch et al., 1986; Jacobs et al., 1972; Wang et al., 2010; Zanella et al., 1989). This closely correlates with the "gold standards" of measuring iron stores in bone marrow or liver biopsy (Peng & Uprichard, 2017).

Given that iron is an essential component for many metabolic processes, the immune system has developed mechanisms for iron sequestration as part of the inflammatory response in order to prevent invading pathogens and tumors from utilizing iron (Wang et al., 2010). Hence, increased levels of SF during the immune system-based acute phase response do not necessarily correlate with iron availability or stores, but rather are a general indicator of inflammation (Dignass et al., 2018). This becomes a critical issue when assessing iron deficiency (ID), as elevations in SF during inflammation can mask the presence of ID (Suchdev et al., 2017). However, this makes the assessment of iron status in the presence of inflammation more complex (Dignass et al., 2018; Knovich et al., 2009; Muñoz, Gomez-Ramirez, et al., 2017). Additionally, the two subunits of ferritin (FTL and FTH) have been reported to differentially locate during periods of inflammation; this complicates the use of these subunits as an inflammatory diagnostic tool (Ahmad et al., 2013). In analyzing data from the Biomarkers Reflecting the Inflammation and Nutritional Determinants of Anemia (BRINDA) project, Suchdev et al. (2017) identified that all their examined indicators of iron status (SF, serum TfR, total body iron) were affected by inflammation, and suggested utilizing C-reactive protein (CRP), a measure of acute inflammation, and α1-acid glycoprotein, a measure of chronic inflammation, in addition to iron indicators to better account for the full range and severity of inflammation.

Extremely elevated SF, in excess of five times the upper limit of normal (Evensen et al., 2007), can indicate adult-onset Still disease. Still disease is a systemic inflammatory disorder that is characterized by fever, arthritis, and rash (Knovich et al., 2009; Zandman-Goddard & Shoenfeld, 2007). More extremely elevated SF (above 10,000 ug/L), especially in the context of autoimmune disorders, such as Still disease and systemic lupus erythematosus (SLE), and viral infections, indicates the possibility of hemophagocytic syndrome (Emmenegger et al., 2001), which involves the phagocytosis of red blood cells by macrophages (Knovich et al., 2009), along with a final common pathway of elevated triglycerides, ferritin, pancytopenia, and highly fatal multiple organ failure (Sekigawa et al., 2001).

Hepcidin regulates serum iron levels by activating the endocytosis and proteolysis of ferroportin, the sole mammalian iron exporter. In healthy individuals, iron status is monitored by hepatocytes, which regulate hepcidin promoter activity according to iron needs. If iron levels are low, iron is released by ferroportin, allowing hepcidin levels to remain low; if iron overload is detected, hepcidin is activated to sequester the excess iron (Ueda & Takasawa, 2018). Unregulated activity of hepcidin can therefore result in hypoferremia due to iron sequestration (Ganz & Nemeth, 2009). Interleukin-6 (IL-6), an inflammatory cytokine, stimulates hepcidin to decrease erythropoiesis due to a lack of bioavailable iron for hemoglobin (Kroot et al., 2011).

No physiologic process is present in the body to excrete excess iron, leaving individuals susceptible to developing iron overload. Iron overload may result from increased absorption, transfusion, or hereditary disease. Excess iron collects within the internal organs, specifically the liver and heart, where it causes chronic free-radical induced injury (Wang et al., 2010). Excess iron may be a symptom or complication of a hereditary disease, such as hereditary hemochromatosis (HH), an autosomal recessive disorder that causes an enhancement in the intestinal absorption of excess iron (Santos et al., 2012). Too much iron in the body can lead to a plethora of problems, including arthritis, skin pigmentation, hypogonadism, cardiomyopathy, and diabetes. The majority of individuals with HH contain mutant hemochromatosis (*HFE*) genotypes, including homozygosity for p.Cys282Tyr or p.Cys282Tyr, and compound heterozygosity for p.His63Asp; based on these results, it is suggested that genetic testing be performed for these mutations in all patients with primary iron overload and an idiopathic increase in transferrin saturation (TSAT) and/or SF values (Santos et al., 2012).

Another genetic disorder characterized by excess iron accumulation is known as neuroferritinopathy (NF). NF was first discovered in 2001 and is a movement disorder identified by excess iron in specific areas of the brain (Lehn et al., 2012). NF is the only known autosomal dominant genetic disease of neurodegeneration caused by mutations in the ferritin light polypeptide 1 (*FTL1*) gene (Keogh et al., 2013; Kumar et al., 2016). The modification causes mutant L-chain ferritins that negatively alter ferritin function and stability (Kuwata et al., 2019; McNally et al., 2019). Several conditions indicative of NF include brain iron accumulation (NBIA) disorder alongside pantothenate kinase-associated neurodegeneration (PKAN), phospholipase A_2-associated neurodegeneration, mitochondrial membrane protein-associated neurodegeneration (MPAN), and beta-propeller protein-associated neurodegeneration (BPAN) (Hayflick et al., 2018). NBIAs are typically characterized by dystonia, Parkinsonism, spasticity, and iron accumulation within the basal ganglia. Depending on the NBIA subtype, the condition may also exhibit hyperphosphorylated tau, axonal swelling, and Lewy body formation (Arber et al., 2016). NF is typically considered as a diagnosis in patients exhibiting movement disorders, decreased SF, variable phenotypes, negative genetic testing for common movement disorders such as Huntington disease, and imaging showing potential iron deposits in the brain (Kumar et al., 2016).

Iron deficiency (ID), referring to a reduced amount of iron stores, is usually an acquired disorder that affects over one billion people worldwide (Camaschella, 2015; Miller, 2013). Inadequate iron intake is often due to poverty, malnutrition, dietary restriction, and malabsorption; additional causes include menstrual periods, gastrointestinal bleeding, and chronic blood loss (DeLoughery, 2017; Kassebaum et al., 2014; Sankaran & Weiss, 2015). SF analysis is the most efficient test for a diagnosis of ID (DeLoughery, 2017). In children, ID is most commonly caused by insufficient dietary iron intake when compared to a child's rapid growth rate, as well as gastrointestinal issues due to cow's milk (Ozdemir, 2015).

It has been reported that more than one in three pregnant individuals present with iron-deficiency anemia worldwide (Lewkowitz & Tuuli, 2019). Anemia in pregnant individuals could affect the fetus' intrauterine growth and may cause neurodevelopmental impairment (Marell et al., 2019). Maternal anemia in early pregnancy is associated with an increased risk of autism spectrum disorder, attention-deficit/hyperactivity disorder, and intellectual disability (Wiegersma et al., 2019). Efficient vitamin and mineral supplementation are vital during pregnancy for the health of both the mother and of the fetus; however, certain supplements may be more helpful than others. It has been suggested that in pregnant women, intravenous iron administration may be a more effective treatment option than oral iron administration (Lewkowitz & Tuuli, 2019).

Analytical Validity

Low SF (<30ug/L) is a sensitive and specific indicator for ID (Dignass et al., 2018). However, a normal SF level can be misleading in the context of inflammation (Peng & Uprichard, 2017). Dignass et al. (2018) published recommendations which stated that the standard ID level is <30 μg/L and that "A serum ferritin threshold of <100 μg/L or TSAT < 20% can be considered diagnostic for iron deficiency in congestive heart failure (CHF), chronic kidney disease (CKD), and inflammatory bowel disease (IBD). If serum ferritin is 100-300 μg/L, TSAT < 20% is required to confirm iron deficiency. Routine surveillance of serum ferritin and TSAT in these at-risk groups is advisable so that iron deficiency can be detected and managed" (Dignass et al., 2018).

Biomarker glycoprotein acetylation (GlycA) has been associated with chronic inflammation and utilizes nuclear magnetic resonance (NMR) to measure the serum or plasma concentration of the *N*-acetyl methyl functional groups of *N*-acetylglucosamine glycans associated with inflammation; these include transferrin, haptoglobin, α_1-acid glycoprotein, α_1-antitrypsin, and α_1-antichymotrypsin (Ritchie et al., 2015). According to Otvos et al. (2015), the simple integration of the GlycA signal to accurately quantify concentration is not possible due to signal overlap with allylic protons of unsaturated fatty acids in the plasma or serum sample; therefore, a linear least-squares deconvolution determination must be performed. In doing so, Otvos et al. (2015) have shown that GlycA has lower imprecision and variability than high-sensitivity C-reactive protein (hsCRP), cholesterol, and triglyceride testing; however, "because the GlycA signals originating from different plasma glycoproteins are not distinguishable, and the glycan on each is heterogeneous and varies dynamically, only a rough estimate can be made of how much each contributes to measured plasma GlycA concentrations" (Otvos et al., 2015). Consequently, the GlycA test cannot be used to accurately determine concentration of individual proteins, including transferrin.

Dahlfors et al. (2015) measured serum hepcidin in more than 400 patients using a competitive ELISA assay; several types of patients were included in this study including those with liver disorders and iron disorders, as well as healthy individuals. The researchers note that this ELISA assay has a good correlation with light chromatography with tandem mass spectroscopy (LC-MS/MS) (r=0.89), but it does cross-react with forms of hepcidin (hepcidin-20 and -22) that are not associated with iron disorder

biomarkers (Dahlfors et al., 2015). Another study by Karlsson (2017) compared the ELISA hepcidin assay to the use of ferritin, C-reactive protein (CRP), and IL-6 to differentiate ID anemia and anemia of inflammation in elder patients. Even though the study was small (n=30), they measured a sensitivity and specificity of the hepcidin assay of 100% and 67%, respectively, as compared to the lower sensitivity but higher specificity of ferritin (91% and 83%, respectively). It was concluded that "Hepcidin shows a strong positive correlation with ferritin, and also correlates positively with C-reactive protein in this patient population" (Karlsson, 2017). Recently, Chen et al. (2019) have developed a high-performance liquid chromatography/tandem mass spectrometry (HPLC/MS/MS) method, in accordance to CLSI-C62A guidelines, to measure serum hepcidin levels. This method has intra- and inter-day coefficients-of-variation (CVs) of <3% and <6%, respectively, with relative error rates ≤1.2% and ≤4.4% at ambient temperature and 4°C, respectively. The authors also report that the relative error rate after three cycles of freeze-thaw (-70°C) is ≤1.8% (Chen et al., 2019).

da Silva et al. (2019) has showed that both iron deficiency anemia (IDA) and sickle cell disease (SCD) can be detected in whole human blood samples via Raman spectroscopy; this study detected both IDA and SCD, when compared to healthy subject controls, with a sensitivity of 93.8% and a specificity of 95.7%. These results were based on detailed spectra analysis methods such as partial least squares and principal component analysis (da Silva et al., 2019).

Gerday et al. (2020) measured urinary ferritin in neonatal intensive care unit (NICU) patients, and found that in those neonates at risk for iron deficiency (n=49), "a corrected urine ferritin < 12 ng/mL had a sensitivity of 82% (95% CI, 67-93%) and a specificity of 100% (CI, 66-100%) for detecting iron-limited erythropoiesis, with a positive predictive value of 100% (CI, 89-100%)." Though iron deficiency can be confirmed via serum iron, transferrin, SF, among other tests, the volume of blood and costs associated with these tests necessitate a non-invasive and accurate alternative for diagnosing iron deficiency (Gerday et al., 2020).

Jones et al. (2021) investigated the effect of delayed processing on measuring 25 micronutrients and select clinical biomarkers, including iron (ferritin), in human blood samples. Blood from 16 healthy participants was collected and processed within either two hours or 24 hours. The concentration difference between the two process delays was compared. All analytes had a 4% or lower change in concentration between the two delays. There was no significant effect of delayed processing on ferritin. The authors concluded that "in blood collected from adult participants, delayed processing of chilled, whole blood for 24 hours did not materially affect the measured concentrations of the majority of micronutrient and selected clinical biomarkers" (Jones et al., 2021).

Bell et al. (2021) performed a meta-analysis to study genes associated with iron homeostasis. Data about blood levels of ferritin, total iron binding capacity, iron saturation, and transferrin saturation was used from three genome-wide association studies from Iceland, the UK, and Demark. The authors identified 56 loci with variants associated with one or more of the biomarkers, 46 of which are novel variants. Specifically, "variants at *DUOX2*, *F5*, *SLC11A2* and *TMPRSS6* associate with iron deficiency anemia, while variants at *TF*, *HFE*, *TFR2* and *TMPRSS6* associate with iron overload" (Bell et al., 2021).

Clinical Utility and Validity

Dysregulated iron metabolism has been implicated in a variety of pathophysiological conditions from mild ID to anemia, iron overload, inflammation, infection, cancer, and cardiovascular and neurodegenerative diseases (Gozzelino & Arosio, 2016). Initial signs and symptoms of iron overload are insensitive and nonspecific, so laboratory studies including ferritin are clinically useful in the

identification and treatment of iron overload (Fleming & Ponka, 2012; Knovich et al., 2009; Koperdanova & Cullis, 2015). According to the Hemochromatosis and Iron Overload Screening (HEIRS) study (McLaren et al., 2003), ferritin levels above 200 ng/mL (449 pmol/L) in women or 300 ng/mL (674 pmol/L) in men with no signs of inflammatory disease warrant additional testing. Therapeutic phlebotomy is indicated in patients with hemochromatosis who have high TSAT and SF levels of more than 1000 ng/mL (2247 pmol/L). Therapeutic phlebotomy is also recommended in patients who do not have anemia (Fleming & Ponka, 2012; Salgia & Brown, 2015; van Bokhoven et al., 2011). Saeed et al. (2015) used a receiver operating characteristic curve to evaluate the value of ferritin >500 ng/mL for diagnosing hemophagocytic lymphohistiocytosis (HLH) in 344 consecutive patients and found that the optimal maximum SF level for the diagnosis of HLH was 3951 ng/mL.

Abioye et al. (2019) collected data from 2,100 pregnant individuals in Tanzania to determine how capable hematologic biomarkers such as hemoglobin and hepcidin were at detecting IDA in pregnant individuals; hepcidin administration >1.6 µg/L was found to reduce the risk of anemia at delivery by an estimated 49%. This study suggests that both hemoglobin and hepcidin may be helpful in determining iron supplementation needs in "resource-limited countries" (Abioye et al., 2019).

Ismail et al. (2019) studied the role of hepcidin in children with b-thalassemia (n = 88 total). The authors measured both serum hepcidin and SF levels as well as determined the hepcidin:ferritin ratio. As expected, serum hepcidin significantly correlated with the hepcidin:ferritin ratio, but the authors reported that there was no statistically significant difference in serum hepcidin levels between splenectomized and non-splenectomized patients. Serum hepcidin levels were more elevated in individuals with beta-thalassemia, especially those with beta-thalassemia major (bTM), than in control patients (21.74 ng/mL and 13.01 ng/mL, respectively). The authors conclude, "Knowing that hepcidin in serum has a dynamic and multi-factorial regulation, individual evaluation of serum hepcidin and follow up, e.g. every six months could be valuable, and future therapeutic hepcidin agonists could be helpful in management of iron burden in such patient" (Ismail et al., 2019).

Yuniati et al. (2019) studied the association between maternal vitamin D, ferritin, and hemoglobin levels during the first trimester of pregnancy, and how these factors affected birthweight. Data collected from these individuals included maternal demography, bloodwork to test ferritin levels, 25(OH) vitamin D results in their first trimester, and the final birthweight of the child after delivery. A total of 203 Indonesian individuals were followed until delivery; it was determined that neither vitamin D, ferritin or hemoglobin levels significantly impacted birthweights in this study. However, the authors suggest that other unknown variables may be at play here and that nutritional supplementation during pregnancy is still important (Yuniati et al., 2019).

Kwiatek-Majkusiak et al. (2020) investigated the connection between hepcidin and chronic neuroinflammation. Serum hepcidin and IL-6 were found to be involved in the progression of Parkinson's Disease. Dysregulation in immune/inflammatory pathways, wherein levels of serum hepcidin and IL-6 would be elevated, were not only predictive of neurodegeneration, with IL-6- induced hepcidin expression in astrocytes, microglia, and epithelial cells, but also response to deep brain stimulation treatment (Kwiatek-Majkusiak et al., 2020).

Brandtner et al. (2020) found linkages between serum markers of iron metabolism and prognosis of sepsis survival. Positive correlations were found between increased serum iron and SF levels and severity of organ failure (SOFA score) and mortality. High TSAT, elevated ferritin and serum iron levels, and low transferrin concentrations were associated with decreased chances of survival as well. This indicates the

utility of iron metabolism in the context of extreme systemic inflammation; from this study, it was also concluded that TSAT can be a stand-alone predictor of sepsis survival (Brandtner et al., 2020).

Nalado et al. (2020) evaluated the diagnostic validity of GDF-15 and hepcidin as biomarkers of IDA in non-dialysis CKD patients. Serum levels of GDF-15 and hepcidin were measured in 312 non-dialysis CKD patients and 184 healthy control participants in Johannesburg, South Africa. For absolute IDA diagnosis among CKD patients, GDF-15 had a predictive value of 74.02%. For functional IDA diagnosis among CKD patients, hepcidin had a predictive value of 70.1%. The authors concluded that "serum GDF-15 is a potential biomarker of absolute IDA, while hepcidin levels can predict functional IDA among CKD patients" (Nalado et al., 2020).

Phillips et al. (2021) studied how the full blood count (FBC) parameters change in older patients. FBC, mean corpuscular volume (MCV), and red cell distribution width (RDW) test results were compiled from male and female patients aged 1-100 years from the National Health Service in England. In males, the mean hemoglobin concentration increased from birth until age 20, then decreased at a steady rate from age 20 to 70, then decreased at a higher rate after age 70. In females, the mean hemoglobin concentration increased from birth until age 14, then decreased slowly from age 14 to 30, then increased again from age 30 to age 60, and then decreased after the age of 60. Overall, "hemoglobin concentrations in males and females begin to converge after age 60 and equalize by approximately 90 years." The authors concluded that FBC parameters trend throughout life, particularly "a falling hemoglobin level and rising MCV and RDW with older age" (Phillips et al., 2021).

Mei et al. (2021) performed a cross-sectional study using data from the US National Health and Nutrition Examination Survey to determine physiologically based SF concentration thresholds for iron deficiency in healthy children (12-59 months) and non-pregnant individuals (15-49 years). The study analyzed the relationship between SF and hemoglobin, and the relationship between SF and soluble transferrin receptor. The study resulted in SF concentration thresholds for iron deficiency of "about 20 µg/L for children and 25 µg/L for non-pregnant women." The authors concluded that "physiologically based thresholds for iron deficiency might be more clinically and epidemiologically relevant than those based on expert opinion" (Mei et al., 2021).

Garcia-Casal et al. (2021) performed a meta-analysis studying the diagnostic accuracy of serum and plasma ferritin concentrations for detecting iron deficiency or overload in primary and secondary iron-loading syndromes. The authors used 72 studies, with a total of 6095 participants, that measured serum or plasma ferritin concentrations. The authors compared ferritin blood tests to iron levels in the bone marrow to diagnose iron deficiency and compared ferritin blood tests to iron levels in the liver to diagnose iron overload. The authors concluded that at a threshold of 30 µg/L, there "is low-certainty evidence that blood ferritin concentration is reasonably sensitive and a very specific test for iron deficiency." Additionally, there is "very low certainty that high concentrations of ferritin provide a sensitive test for iron overload in people where this condition is suspected." The authors note that overall confidence in the studies is low because of potential bias, indirectness, and heterogenous evidence, and that there is insufficient evidence to make conclusions about using ferritin concentrations to diagnose iron deficiency or overload in asymptomatic people (Garcia-Casal et al., 2021).

Auerbach et al. (2021) performed a study to assess the accuracy of diagnosing IDA using the complete blood cell count (CBC) and reticulocyte hemoglobin equivalent (RET-He) analysis. 556 patients referred for the diagnosis and/or treatment of anemia were studied at baseline, and 150 of the participants were later studied after intravenous iron treatment. RET-He identified iron deficiency with a 68.2% sensitivity and 69.7% specificity. RET-He predicted responsiveness to intravenous iron with 84% sensitivity and 78%

specificity. The authors concluded that "CBC and RET-He can identify patients with IDA, determine need for and responsiveness to intravenous iron, and reduce time for therapeutic decisions" (Auerbach et al., 2021).

Tahara et al. (2022) examined the usage of RET-He as a marker of iron deficiency in patients with heart failure, as both anemia and iron deficiency are common among patients with heart failure. RET-He has been considered as a proxy due to the limitations of using serum ferritin and transferrin saturation for the diagnosis of iron deficiency in the clinical setting. Namely, ferritin can be overestimated in cases of chronic inflammation, such as in the case of heart failure, and thus may be inaccurately measured for the diagnosis of iron deficiency. In this prospective study, researchers enrolled 142 patients hospitalized for decompensated heart failure, with 65% of them having iron deficiency. RET-He was directly correlated with serum iron and ferritin concentrations and TSAT for iron deficiency. They found that "there was a poor relationship between quartile of RET-He and [heart failure] hospitalization or death but increases or decreases in RET-He between admission and discharge were associated with a worse outcome." This demonstrated a potential for using RET-He for predicting improvements in iron deficiency per response to IV iron and prognosis of patients with comorbid iron deficiency and heart failure (Tahara et al., 2022).

Guidelines and Recommendations

Guidelines and recommendations related to the screening of anemia in certain populations are available; however, published recommendations regarding the use of ferritin as a first-line test in asymptomatic individuals have not been identified.

Regarding NF, "At present, no established guidelines or specific management recommendations for patients with NF have been identified. An individualized symptomatic approach to treatment is recommended" (Kumar et al., 2016). To date, the only NBIA guidelines published concerning diagnosis and management of the condition is pantothenate kinase-associated neurodegeneration (PKAN, formerly called Hallervorden-Spatz syndrome) (Hogarth et al., 2017).

American Gastroenterological Association (AGA)

The AGA has published its official recommendations on the gastrointestinal evaluation of iron deficiency anemia (IDA). It has stated:

- "In patients with anemia, the AGA recommends using a cutoff of 45 ng/mL over 15 ng/mL when using ferritin to diagnose iron deficiency. Strong recommendation, high-quality evidence. Comment: In patients with inflammatory conditions or chronic kidney disease, other laboratory tests such as C-reactive protein, transferrin saturation, or soluble transferrin saturation, may be needed in conjunction with ferritin to diagnose iron deficiency anemia" (Ko et al., 2020).

American Society of Clinical Oncology (ASCO) and the American Society of Hematology (ASH)

The ASCO and ASH have published guidelines regarding the management of cancer-related anemia with erythropoiesis-stimulating agents (ESAs). It is stated that "With the exception of selected patients with MDS, ESAs should not be offered to most patients with nonchemotherapy-associated anemia. During ESA treatment, hemoglobin may be increased to the lowest concentration needed to avoid transfusions. Iron replacement may be used to improve hemoglobin response and reduce RBC transfusions for patients receiving ESA with or without ID. Baseline and periodic monitoring of iron, total iron-binding capacity, transferrin saturation, or ferritin levels is recommended" (Bohlius et al., 2019).

American Academy of Family Physicians (AAFP)

The AAPF have recommend the following with "C" evidence ratings (consensus, disease-oriented evidence, usual practice, expert opinion, or case series):

- "A low serum ferritin level is associated with a diagnosis of iron deficiency anemia,"
- "Older patients with suspected iron deficiency anemia should undergo endoscopy to evaluate for occult gastrointestinal malignancy," and
- "Low-dose formulations of iron (15 mg of elemental iron) can be effective for treatment of suspected iron deficiency anemia and have a lower risk of adverse effects than standard preparations" (Lanier et al., 2018).

Also stated is: "Patients with an elevated serum ferritin level or macrocytic anemia should be evaluated for underlying conditions, including vitamin B12 or folate deficiency, myelodysplastic syndrome, and malignancy" (Lanier et al., 2018).

In 2021, the AAFP also published the 2020 AGA guidelines on iron deficiency anemia, reported above (please see the guidelines for the AGA).

American College of Gastroenterology (ACG)

The ACG practice guidelines regarding the evaluation of abnormal liver chemistries recommend that "All patients with abnormal liver chemistries in the absence of acute hepatitis should undergo testing for hereditary hemochromatosis with an iron level, transferrin saturation, and serum ferritin [Strong recommendation, very low level of evidence]" (Kwo et al., 2017).

World Health Organization (WHO)

The WHO guideline on the use of ferritin concentrations to assess iron status in individuals and populations, published in 2020, updated the previous serum ferritin levels recommendations. The guidelines recommend cut-off serum ferritin levels for iron deficiency in infants (0-23 months) and preschool children (24-59 months) as under 12 µg/L in apparently healthy individuals and under 30 µg/L in individuals with infections or inflammation. The guidelines recommend cut-off serum ferritin levels for iron deficiency in school age children (5-12 years), adolescents (13-19 years), adults (20-59 years), and older persons (over 60 years) as under 15 µg/L in apparently healthy individuals and under 70 µg/L in individuals with infections or inflammation. The guidelines recommend cut-off serum ferritin levels for iron deficiency in apparently healthy pregnant women in their first trimester as under 15 µg/L.

The guidelines recommend cut-off serum ferritin levels for risk of iron overload in school age children (5-12 years), adolescents (13-19 years), adults (20-59 years), and older persons (over 60 years) as over 150 µg/L in apparently healthy individuals females, over 200 µg/L in apparently healthy males, and over 500 µg/L in individuals with infections or inflammation (WHO, 2020).

International Consensus Guideline for Clinical Management of Pantothenate Kinase-Associated Neurodegeneration (PKAN)

An international group released guidelines concerning the clinical management of the NBIA condition PKAN in 2017. Although no specific recommendation is directly given regarding measurement of iron, Hogarth et al. (2017) state, "The role that iron plays in PKAN pathogenesis is still unclear because iron dyshomeostasis is a secondary phenomenon in this disorder. Nevertheless, high iron levels develop in

globus pallidus and probably contribute to cell and tissue damage. The utility of iron chelators has been limited by systemic iron depletion. Newer agents more readily cross the blood-brain barrier yet have a lower affinity for iron, thereby minimizing systemic iron loss." Concerning diagnosis of PKAN, "People suspected to have PKAN based on clinical features should undergo brain MRI using iron sensitive sequences such as SWI, GRE, T2* as a first line diagnostic investigation to identify the characteristic changes. The MRI abnormality, called the 'eye-of-the-tiger' sign, is observed on T2-weighted imaging and consists of hypointense signal in the globus pallidus surrounding a region of hyperintense signal" (Hogarth et al., 2017).

International Consensus Statement on the Peri-operative Management of Anemia and Iron Deficiency

An expert workshop, including several experienced researchers and clinicians, was conducted to develop a guidance for the diagnosis and management of anemia in surgical patients. A series of best-practice and evidence-based statements to advise on patient care with respect to anemia have been published via this workshop. It was stated that serum ferritin measurement is the most sensitive and specific test used for the identification of absolute iron deficiency (Muñoz, Acheson, et al., 2017).

International Consensus Conference on Anemia Management in Surgical Patients (ICCAMS)

The ICCAMS recommends the following for the diagnosis of anemia:

- All patients with anemia should be evaluated for the cause of anemia—wherever possible, early enough preoperatively to enable sufficient time for treatment to be successful.
- It is important to identify iron deficiency, including in patients with anemia of inflammation (or anemia of chronic disease).
- Patients with IDA should be evaluated for the cause of the iron deficiency, whereas patients with anemia and normal iron studies should be evaluated for coexisting causes of anemia (ie, renal disease, primary hematologic disease, and nutrition deficiency).
- Evaluation for iron deficiency should include iron studies (serum iron, total iron binding capacity, transferrin saturation (TSAT), serum ferritin); if available, reticulocyte Hb content and/or serum hepcidin should be considered in inflammatory states.

The most important criteria for defining absolute iron deficiency were ferritin <30 ng/mL and/or TSAT <20%; ferritin <100 ng/mL may define iron deficiency in inflammatory states. If available, either a reticulocyte Hb <29 pg or a serum hepcidin level <20 µg/L also suggest the presence of iron deficiency in inflammatory states (Shander et al., 2023).

European Crohn's and Colitis Organisation (ECCO)

The ECCOguidelines published in 2015 concerning iron deficiency and anemia in IBD with an EL 5-recommendation state, "For laboratory screening, complete blood count, serum ferritin, and C-reactive protein [CRP] should be used. For patients in remission or mild disease, measurements should be performed every 6 to 12 months. In outpatients with active disease such measurements should be performed at least every 3 months" (Dignass et al., 2015). Also mentioned in the section concerning the workup for anemia with an EL-4 recommendation is that anemia workups "should be initiated if the hemoglobin is below normal. The minimum workup includes red blood cell indices such as red cell distribution width [RDW] and mean corpuscular volume [MCV], reticulocyte count, differential blood cell count, serum ferritin, transferrin saturation [TfS], and CRP concentration. More extensive workup includes serum concentrations of vitamin B, folic acid, haptoglobin, the percentage of hypochromic red

cells, reticulocyte hemoglobin, lactate dehydrogenase, soluble transferrin receptor, creatinine, and urea" (Dignass et al., 2015).

Regarding the management of iron deficiency in patients with IBD, ECCO explains that "In patients with IBD the usage of ferritin is complicated by the fact that it is an acute phase protein and can increase in the setting of inflammation," but "if serum ferritin is below the lower cutoff, iron deficiency can be diagnosed, but if ferritin is normal, iron deficiency cannot be excluded in patients with IBD." Consequently, "The 2015 ECCO guidelines therefore recommend a serum ferritin 30 µg/liter as a cutoff in patients with clinical, endoscopical and biochemical remission. In patients with active inflammation a serum ferritin 100 µg/liter may still be consistent with iron deficiency"(Niepel et al., 2018).

The United States Preventive Services Task Force (USPSTF)

The USPSTF states that "the current evidence is insufficient to assess the balance of benefits and harms of screening for iron deficiency anemia in pregnant [individuals] to prevent adverse maternal health and birth outcomes; the current evidence is insufficient to assess the balance of benefits and harms of routine iron supplementation for pregnant [individuals] to prevent adverse maternal health and birth outcomes; the current evidence is insufficient to assess the balance of benefits and harms of screening for iron deficiency anemia in children ages 6 to 24 months" (Siu, 2015a, 2015b). All recommendations have been given a grade I. The screening guidelines for iron deficiency anemia in pregnant individuals are currently being updated as of June 30, 2022.

American Society of Hematology (ASH)

In the ASH "Guidelines for Quantifying Iron Overload", it is stated that "Despite improved availability of advanced imaging techniques, serum ferritin remains the mostly commonly used metric to monitor iron chelation therapy and remains the sole metric in many countries. Serum ferritin measurements are inexpensive and generally correlate with both total body iron stores and clinical outcomes…Given interpatient and temporal variability of serum ferritin values, serum ferritin is best checked frequently (every 3-6 weeks) so that running averages can be calculated; this corrects for many of the transient fluctuations related to inflammation and liver damage." Regarding the use of transferrin, the guidelines also state that "Iron that is bound to transferrin is not redox active, nor does it produce extrahepatic iron overload. However, once transferrin saturations exceed 85%, non-transferrin-bound iron (NTBI) species begin to circulate, creating a risk for endocrine and cardiac iron accumulation. A subset of NTBI can catalyze Fenton reactions and is known as labile plasma iron (LPI). Therefore, transferrin saturation, NTBI, and LPI are potentially attractive serum markers for iron toxicity risk. Transferrin saturation is widely available, but values cannot be interpreted if iron chelator is present in the bloodstream, so patients have to be instructed to withhold iron chelation for at least one day before measurement… Although some studies link elevated LPI to cardiac iron accumulation, large validation studies are lacking. Therefore, to date, these metrics remain important and interesting research tools, but are not suitable for routine monitoring" (Wood, 2014). Within the conclusion of the paper, the author notes that "Serum markers of somatic stores (ferritin and transferrin saturation) are useful surrogates for total iron stores and extrahepatic risk, respectively. However, they cannot replace LIC or cardiac T2* assessment for monitoring chelator efficacy or stratifying end organ risk" (Wood, 2014).

The National Kidney Foundation-Kidney Disease Outcomes Quality Initiative (NKF-KDOQI)

The National Kidney Foundation-Kidney Disease Outcomes Quality Initiative (KDOQI) published guidelines in 2012. In 2013, the Kidney Disease: Improving Global Outcomes (KDIGO) group reviewed

these guidelines in a separate publication. Based on the suggestions made by the KDOQI, the KDIGO "continued to recommend the use of serum ferritin concentration and transferrin saturation (TSAT) to define iron stores and iron availability. For all their imperfections, these metrics remain our best routinely available tools to assess iron status and manage iron supplementation. In the absence of superior, cost-effective, and easily applicable alternatives, this approach seems reasonable" (Kliger et al., 2013).

Further, the KDOQI stated that ferritin testing along with TSAT as part of the evaluation of iron status in individuals with chronic kidney disease who are being treated for anemia is recommended. Also, in agreement with KDIGO, the KDOQI recommend testing prior to initiation of treatment, once per month during initial treatment, and at least every three months after a stable hemoglobin level is reached.

Kidney Disease Improving Global Outcomes (KDIGO)

In the *2012 KDIGO Clinical Practice Guideline for Anemia in Chronic Kidney Disease* publication, a complete blood count, absolute reticulocyte count, serum ferritin, serum transferrin saturation (TSAT), serum vitamin $B_{12,}$ and serum folate levels are recommended as part of an initial evaluation of anemia for all CKD patients, regardless of age or stage of degree progression. Moreover, for patients undergoing ESA therapy, "including the decision to start or continue iron therapy," both TSAT and ferritin should be tested at least every three months; TSAT and ferritin should be tested "more frequently when initiating or increasing ESA dose, when there is blood loss, when monitoring response after a course of IV iron, and in other circumstances where iron stores may become depleted" (KDIGO, 2012).

International Society of Nephrology (ISN)

The most recent guidelines from the ISN, released in 2008, state that for CKD patients "who require iron and/or ESA therapy, measurement of serum ferritin and transferrin saturation every 1-3 months is reasonable, depending upon the clinical status of the patient, the hemoglobin response to iron supplementation, the ESA dose, and recent iron status test results; in stable patients with mild anemia (hemoglobin >110 g/l) who are not receiving iron or ESA therapy, assessment of iron status could be performed less frequently, potentially on a yearly basis" (Madore et al., 2008).

Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
82728	Ferritin
83540	Iron
83550	Iron binding capacity
84466	Transferrin
84999	Unlisted chemistry procedure
0024U	Glycosylated acute phase proteins (GlycA), nuclear magnetic resonance spectroscopy, quantitative Proprietary test: GlycA Lab/Manufacturer: Laboratory Corporation of America
0251U	Hepcidin-25, enzyme-linked immunosorbent assay (ELISA), serum or plasma Proprietary test: Intrinsic Hepcidin IDx™ Test Lab/Manufacturer: IntrinsicDx

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

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Revision History

Revision Date	Summary of Changes
12/04/2024	Reviewed and Updated: Updated the background, guidelines and recommendations, and evidence-based scientific references. Literature review did

	not necessitate any modifications to coverage criteria. The following edits were made for clarity and consistency: Removed "(using serum iron and serum iron binding capacity measurements)" from CC2. Now reads: "2) Measurement of serum transferrin saturation MEETS COVERAGE CRITERIA in any of the following:" Edited CC3 to clarify that testing outside of conditions addressed above is not allowed, including the testing of asymptomatic individuals (individuals should be symptomatic for indications provided in criteria, not just symptomatic in general). Now reads: "3) For all other situations not addressed above, measurement of ferritin or transferrin levels, including transferrin saturation, DOES NOT MEET COVERAGE CRITERIA."

Epithelial Cell Cytology in Breast Cancer Risk Assessment

Policy Number: AHS – G2059 – Epithelial Cell Cytology in Breast Cancer Risk Assessment	Prior Policy Name and Number, as applicable:
Initial Presentation Date: 09/18/2015 Revision Date: February 1, 2025	

Policy Description

Nipple aspiration and/or ductal lavage are non-invasive techniques to obtain epithelial cells for cytological examination to aid in the evaluation of nipple discharge for breast cancer risk (Golshan, 2022). Fine needle aspiration (FNA) is another approach that can be used in the initial diagnosis of a suspicious breast mass, although core biopsy is superior in sensitivity, specificity, and correct histological grading (Moy et al., 2017).

Related Policies

Policy Number	Policy Title
AHS-G2124	Serum Tumor Markers for Malignancies
AHS-M2126	Use Of Common Genetic Variants (Single Nucleotide Polymorphisms) To Predict Risk of Non-Familial Breast Cancer

Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

1) Cytologic analysis of epithelial cells to assess breast cancer risk and manage patients at high risk of breast cancer **DOES NOT MEET COVERAGE CRITERIA**.

Table of Terminology

Term	Definition
ACR	American College of Radiology
ASBS	American Society of Breast Surgeons
CMS	Centers for Medicare and Medicaid Services
DHEA	Dehydroepiandrosterone
FDA	Food and Drug Administration
FNA	Fine needle aspiration
LDT	Laboratory developed Tests
NAF	Nipple aspirate fluid
NCCN	National Comprehensive Cancer Network
PED	Proliferative epithelial disease

Scientific Background

Breast cancer is the most frequently diagnosed cancer and is a leading cause of cancer death in the United States. Nipple discharge is a common breast complaint. Most nipple discharge is of benign origin; however, it is necessary to differentiate patients with benign nipple discharge from those who have an underlying pathology. In approximately five to 15 percent of pathologic nipple discharge cases, cancer is identified (Golshan, 2022).

Breast cancer originates in breast epithelium and is associated with progressive molecular and morphologic changes. Individuals with atypical breast ductal epithelial cells have an increased relative risk of breast cancer. Cytological evaluation of epithelial cells in nipple discharge has been used as a diagnostic aid. Due to the scant cellularity of specimens obtained by expression or aspiration of nipple discharge, ductal lavage was developed to enhance the ease and efficiency of collecting breast epithelial cells for cytologic analysis. The analysis of breast-specific liquid biopsies, such as nipple aspirate fluid, has potential to be used as a biomarker profiling technique for monitoring breast health (Shaheed et al., 2018). Researchers report that the measurement of nipple aspirate fluid, including miRNA, pathological nipple discharge, and breast ductal fluids, may help to improve early detection and management of breast cancer (Moelans et al., 2019).

Fine needle aspiration (FNA) is a biopsy option for a suspicious palpable breast mass. FNA is a rapid diagnosis technique, but it is not as accurate as core needle biopsy. FNA cannot differentiate in situ and invasive cancer and has higher rates of negative results and insufficient samples than core needle biopsy. The success of FNA results also varies with the operator and cytopathologist (Joe & Esserman, 2023).

Analytic Validity

In a retrospective study of 618 patients with nipple discharge over a 14-year period, the sensitivity and specificity of cytology were 17 and 66 percent, respectively; the authors concluded that "nipple discharge cytology has little complementary diagnostic value" (Kooistra et al., 2009).

Clinical Utility and Validity

Hornberger et al. (2015) performed a meta-analysis on the use of nipple aspirate fluid (NAF) in identifying breast cancer based on proliferative epithelial disease (PED). The authors reviewed 16 articles, 20808 unique aspirations, and 17378 subjects. Among cancer-free patients, 51.5% aspirations contained fluid, of which 27.7% showed a PED on cytology. Of the two prospective studies of 7850 women, patients with abnormal cytology showed a 2.1-fold higher risk of developing breast cancer compared to those without fluid (Hornberger et al., 2015).

Chatterton et al. (2016) measured sex steroid levels in nipple aspirate fluid; hormones were measured in samples from 160 breast cancer cases and 157 controls. Results showed a significantly higher concentration of dehydroepiandrosterone (DHEA) in the nipple aspirate fluid of patients with breast cancer compared to controls; further, DHEA levels were highly correlated with estradiol levels, indicating "a potentially important role of this steroid in breast cancer risk" (Chatterton et al., 2016).

Kamalı and Kamalı (2022) studied the usefulness of testing methods in surgical decision making. The study included 141 patients with pathological nipple discharge who were planning to undergo surgery. The diagnostic efficiency of ductal lavage cytology was compared to that of ultrasonography, mammography, magnetic resonance imaging, and ductography. The sensitivity of ductal lavage cytology was 70.5% and the specificity was 94.1%. The authors conclude that "negative cytology does not exclude the possibility of malignancy, and positive results do not help in the differential diagnosis" (Kamalı & Kamalı, 2022).

Guidelines and Recommendations

American Society of Breast Surgeons (ASBS)

The Official Statement by the American Society of Breast Surgeons (ASBS, 2019) regarding Screening Mammography does not mention ductal lavage at all in their statement.

In 2016, the ASBS published a consensus guideline on the concordance assessment of image-guided breast biopsies and the management of borderline or high-risk lesions. These guideline state that "The decision to excise a papillary lesion without atypia needs to be individualized based on risk, including such criteria as size; symptomatology, including palpability and presence of nipple discharge; and breast cancer risk factors" (ASBS, 2016). This is the only mention of nipple discharge in the document.

National Comprehensive Cancer Network (NCCN)

National Comprehensive Cancer Network Clinical Practice Guidelines in breast cancer screening and diagnosis (NCCN, 2023) state that "thermography and ductal lavage are not recommended by the NCCN Panel for breast cancer screening or diagnosis." The NCCN also notes that "the FDA has issued a safety alert stating that ductal lavage should not be a replacement for mammograms" (NCCN, 2023).

Food and Drug Administration (FDA)

In 2017 the FDA issued a safety warning (FDA, 2017) stating that "…the FDA is unaware of any valid scientific data to show that a nipple aspirate test, when used on its own, is an effective screening tool for any medical condition, including the detection of breast cancer or other breast disease."

American College of Radiology (ACR)

The 2022 ACR appropriateness criteria for the evaluation of nipple discharge do not mention cytology. The ACR states that "image-guided FNA and core biopsy are not required for the evaluation of physiologic nipple discharge" but "image-guided FNA and core biopsy are not required for the evaluation of physiologic nipple discharge". The ACR also notes "although some institutions demonstrate good results using FNA, larger series have shown that core biopsy is superior to FNA in terms of sensitivity, specificity, and correct histologic grading of a lesion" (Sanford et al., 2022).

Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
88108	Cytopathology, concentration technique, smears and interpretation (eg, Saccomanno technique)
88112	Cytopathology, selective cellular enhancement technique with interpretation (eg, liquid based slide preparation method), except cervical or vaginal
88172	Cytopathology, evaluation of fine needle aspirate; immediate cytohistologic study to determine adequacy for diagnosis, first evaluation episode, each site
88173	Cytopathology, evaluation of fine needle aspirate; interpretation and report
88177	Cytopathology, evaluation of fine needle aspirate; immediate cytohistologic study to determine adequacy for diagnosis, each separate additional evaluation episode, same site (List separately in addition to code for primary procedure)

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

Evidence-based Scientific References

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Revision History

Revision Date	Summary of Changes

09/06/2023	Reviewed and Updated: Updated the background, guidelines and recommendations, and evidence-based scientific references. Literature review necessitated the following changes to coverage criteria: CC1 updated to reflect that all cytological analysis for breast cancer diagnosis DNMCC, as biopsy should be used to diagnose. CC now reads: "1) Cytologic analysis of epithelial cells to assess breast cancer risk and manage patients at high risk of breast cancer DOES NOT MEET COVERAGE CRITERIA." Added CPT code 88172, 88173, 88177

Evaluation of Dry Eyes

Policy Number: AHS – G2138 – Evaluation of Dry Eyes	Prior Policy Name and Number, as applicable:
Initial Presentation Date: 09/19/2016 Revision Date: 03/06/2024	

Policy Description

Dry eye disease (dysfunctional tear syndrome, DED) is defined by the Dry Eye Workshop II as "a multifactorial disease of the ocular surface characterized by a loss of homeostasis of the tear film, and accompanied by ocular symptoms, in which tear film instability and hyperosmolarity, ocular surface inflammation and damage, and neurosensory abnormalities play etiological roles" (Craig, Nichols, et al., 2017). Five to fifteen percent of the United States population suffers from dry eye disease, leaving a substantial burden on functional vision, general health status, and workplace productivity (Dana et al., 2020).

Related Policies

Policy Number	Policy Title
AHS-M2083	Genetic Testing for Ophthalmologic Conditions

Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) For individuals suspected of having dry eye, testing of tear osmolarity **MEETS COVERAGE CRITERIA** in **any** of the following situations:

   a) To help determine the severity of dry eye disease.

   b) To monitor effectiveness of therapy.

2) For individuals suspected of having dry eye disease based on comprehensive eye examination, testing for MMP-9 protein in human tears **DOES NOT MEET COVERAGE CRITERIA** .

3) For individuals suspected of having dry eye disease, testing for lactoferrin and/or IgE **DOES NOT MEET COVERAGE CRITERIA**.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

4) For individuals suspected of having dry eye disease, all other testing not discussed above **DOES NOT MEET COVERAGE CRITERIA**.

Table of Terminology

Term	Definition
AAO	American Academy of Ophthalmology
AAOPT	American Academy of Optometry
AOA	American Optometric Association
ASCRS	American Society of Cataract and Refractive Surgery
ATD	Advanced tear diagnostics
CA-6	Carbonic anhydrase-6
CLIA '88	Clinical Laboratory Improvement Amendments of 1988
CMS	Centers for Medicare and Medicaid Services
DED	Dry eye disease
DEWS	Dry eye workshop
DTS	Dysfunctional tear syndrome
FDA	Food and Drug Administration
IgA	Immunoglobulin A
IgE	Immunoglobulin E
IgG	Immunoglobulin G
IgM	Immunoglobulin M
LASIK	Laser-assisted in situ keratomileusis
LDTs	Laboratory-developed tests
MMP	Matrix metallopeptidase
MMP-9	Matrix metalloproteinase-9
NIKBUT	Non-invasive tear breakup time
OSD	Ocular surface disorders
OSDI	Ocular surface disease index

OSS	Ocular surface staining
PSP	Parotid secretory protein
SP-1	Salivary protein-1
SPEED	Standard patient evaluation of eye dryness
TBUT	Tear break-up time
TFBUT	Tear film break-up time
TFOS	Tear film & ocular surface
VAS	Visual analogue scale

Scientific Background

Tears are necessary for maintaining the health of the inner and outer surfaces of the eyelid and for providing clear vision. The tear film of the eye consists of aqueous, mucous, and lipid components. A healthy tear film is necessary for protecting and moisturizing the cornea, as well as for providing a refracting surface for light entering the eye (Willcox et al., 2017). Dysfunction of any component of the tear film can lead to dry eye disease (dysfunctional tear syndrome, DED). Dry eye is a common and often chronic problem, particularly in older adults as age affects the entire lacrimal functional unit (Ezuddin et al., 2015). The exact prevalence of dry eye is unknown due to difficulty in defining the disease and the lack of a single diagnostic test to confirm its presence, but the 2013 National Health and Wellness Survey estimated the rate of dry eye in the United States to be 6.8%, or about 16.4 million people; prevalence tended to increase with age, with the 18-34 age group only comprising 2.7% of the total and the 75+ age group comprising 18.6% (Farrand et al., 2017; Shtein, 2024). Risk factors for dry eye include increasing age, systemic comorbidities such as diabetes and autoimmune disease, and therapeutic treatments for anxiety, depression, and sleep disorders (Periman, 2020).

Further, the 2017 Tear Film & Ocular Surface (TFOS) Society International Dry Eye Workshop (DEWS) II reported that "the core mechanism of dry eye disease is tear hyperosmolarity, which is the hallmark of the disease" (Craig, Nichols, et al., 2017).

Dry eye is classified into two general groups: decreased tear production and increased evaporative loss. Decreased tear production may lead to hyperosmolarity of the tear film and inflamed ocular surface cells. An age-related ductal obstruction is the most common cause of decreased tear production. Increased evaporative loss is typically caused by problems in the Meibomian gland when the glands that produce the lipid portion of the tear film fail. This lipid portion normally allows the tear film to spread evenly, minimizing evaporation. In both groups, tear film hyperosmolarity and subsequent ocular surface inflammation lead to the variety of symptoms and signs associated with dry eye (Shtein, 2024).

Most patients will present with symptoms of chronic eye irritation, such as red eyes, light sensitivity, blurred vision, or unusual sensations (gritty, burning, foreign, etc.). However, significant variability in the patient-reported symptoms and signs, as well as a lack of correlation between these symptoms and signs, make it difficult to diagnose dry eye, and no single definitive test to diagnose dry eye exists. Dry eye is typically diagnosed with a combination of patient symptoms and physical findings, such as reduced blink rate or eyelid malposition (Shtein, 2024). Incomplete blinking may also be considered for mild-to-moderate dry eye assessment (Jie et al., 2019). Further, visual acuity was found to be particularly poor in those with vision-related symptoms due to dry eyes (Szczotka-Flynn et al., 2019).

The primary way to treat dry eye is artificial tears, although corticosteroids, topical cyclosporine A, or anti-inflammatories such as Lifitegrast ophthalmic solution five percent may be used to supplement treatment. Avoiding environmental factors, such as heavy smoke or dry heating air, is also recommended (Messmer, 2015). It was recently reported by Holland et al. (2019), who reviewed two decades worth of data on the safety and efficacy of controlled topical ophthalmic drug administration for DED treatment, that poor standardization of endpoints across studies causes challenges in the improvement of this field. However, recent advances in drug delivery and a greater understanding of DED will assist in the improvement of ophthalmic drugs.

Accurate diagnosis of dry eye disease requires a variety of tests including patient-reported symptom questionnaires, tear film break-up time (TFBUT), Schirmer test, ocular surface staining, and meibomian gland functionality. However, many of these tests lack consistency and reliability in diagnosis. New tools have been developed which allow for the quantification of tear film characteristics including measurement of tear osmolarity and measurement of inflammatory mediators such as matrix metallopeptidase enzymes, and biomarkers such as lactoferrin (Shtein, 2024).

Tear Osmolarity

Osmolarity is a measurement of the concentration of dissolved solutes in a solution. Hyperosmolarity of the tear film is a recognized and validated marker of dry eye. The following tear osmolarity thresholds have been suggested for establishing the severity of dry eyes: 270-308 mOsm/L for normal eyes, 308-316 mOsm/L for mild dry eye, and >316 mOsm/L for moderate to severe dry eye (Milner et al., 2017). Tomlinson et al. (2006) suggested a cut-off of 316 mOsm/L, but the sensitivity was found to be 0.59 when applied to the independent sample described in the study. Furthermore, decreasing the cut-off to increase the sensitivity decreased the specificity and overall accuracy significantly. Overall, the overlap between normal and dry eyes contributes heavily to the difficulty in establishing a cut-off (Tomlinson et al., 2006). Some studies suggest that osmolarity shows the strongest correlation with severity of dry eye based on the metrics used, but at the same time lack correlation to other objective signs of dry eye. In general, tear osmolarity results vary between clinical signs and symptoms, which can make them difficult to interpret (Akpek et al., 2019).

The test "TearLab" is based on assessment of the osmolarity of tears. TearLab collects a 50 µL tear sample, analyzes its electrical impedance, and provides an assessment of the osmolarity of the sample and thereby the tear (Willcox et al., 2017). Baenninger et al. (2018) completed an extensive systematic review investigating 1362 healthy eyes of participants from 33 different studies; this review found a weighted mean osmolarity of 298 mOsm/L via the TearLab test. Final comments from the researchers highlighted the great variability of osmolarity measurements that were found with the TearLab system, suggesting caution when interpreting TearLab osmolarity results (Baenninger et al., 2018).

Matrix Metallopeptidase (MMP) Enzymes

Inflammation is a common factor across the subtypes of DED. Levels of inflammatory mediators, such as cytokines, may be assessed in the tear film. For example, the matrix metallopeptidase (MMP) enzymes play an important role in wound healing and inflammation by degrading collagen. Elevated levels of MMP-9, a member of the MMP family produced by corneal epithelial cells (Chotikavanich et al., 2009; Honda et al., 2010), have been observed in the tears of patients with dry eye (Sambursky et al., 2013). A study with 101 patients with DED and controls (54 controls, 47 with DED) was performed to assess

correlation of the protein MMP-9 with dry eye. All 101 underwent MMP-9 testing of the tear film and were evaluated for symptoms and signs of DED. The tear film was then analyzed for MMP-9 by InflammaDry, which detects MMP-9 levels of more than 40 ng/mL. The MMP-9 results were positive in 19 of the 47 dry eye patients (40.4%) and three of the 54 controls (5.6%). The authors concluded that "MMP-9 correlated well with other dry eye tests and identified the presence of ocular surface inflammation in 40% of confirmed dry eye patients," and suggested it may be helpful to identify patients with autoimmune disease and ocular surface inflammation (Messmer et al., 2016). The American Academy of Ophthalmology (AAO) has noted MMP-9 does not differentiate dry eye from any other inflammatory ocular surface disease and does not include this test in its appendix on diagnostic tests (Akpek et al., 2019).

Lactoferrin

Another biomarker associated with inflammation is lactoferrin. Lactoferrin is thought to promote the healing process resulting from inflamed dry eyes and is used to assess the lacrimal glands (Willcox et al., 2017). "Lactoferrin & Dry Eye Disease (DED): The measurement of ocular lactoferrin, as a biomarker, has long been established and accepted as the "medical standard" in assessing the secretory function of the lacrimal gland. Low lactoferrin levels directly correlate to aqueous deficiency. Normal lactoferrin levels indicate normal lacrimal gland function" (AXIM, 2021). The AXIM Eye Lactoferrin test has a specificity of 98%, and a sensitivity of 83%. A High Lf: > 1.0 mg/ml and a Low Lf: < 0.8 mg/ml (AXIM, 2021).

Additional Tests

Other tests noted by the American Academy of Optometry (AAOPT) are the tear break-up time test, the ocular surface dry staining test, the Schirmer test, and the fluorescein dye disappearance test. The tear break-up time test evaluates the precorneal tear film's stability with a fluorescein dye, which is inserted in the lower eyelid. If the tear film layer develops a dark discontinuity (usually blue) in under ten seconds, the result is considered abnormal. The ocular surface dry staining test stains areas of discontinuity of the corneal epithelial surface, which may contribute to dryness. A fluorescein dye is typically used, although a rose bengal dye or a lissamine green dye may be used as well. The Schirmer test quantifies the amount of tears produced by each eye. This is done by placing small strips of filter paper in the lower eyelid and checking the length (in mm) of wet strips in a certain amount of time. This test is noted as an extremely variable test, so it should not be used as the only diagnostic test. Finally, the fluorescein dye disappearance test places a certain amount of fluorescein dye on the ocular surface, and then evaluates how much of that dye was cleared from the surface (Akpek et al., 2019; Shtein, 2024).

Evaluation of dry eyes is difficult for numerous reasons. Currently, no "gold standard" or globally accepted guideline for diagnosis of dry eye exists, and no threshold between healthy and affected eyes has been established. Many other features of testing (repeatability, high variability, including highly variable sensitivity and specificity of tests and dependence on clinical conditions) and the disease itself—its multifactorial status, examiner subjectivity, reliance on patient-based questionnaires, for example—make diagnosis of dry eye especially challenging (Kanellopoulos & Asimellis, 2016). Despite promising sensitivities, specificities, or other strong statistical findings, these numbers should still be considered in the context of clinical findings (Akpek et al., 2019).

Clinical Utility and Validity

Tear Osmolarity

Brissette et al. (2019) measured the utility of the TearLab test in 100 patients with DED-like symptoms who had normal tear osmolarity results. This study aimed to use the test to identify diagnoses other than DED. All patients included in the study had a normal tear osmolarity test (<308 mOsm/L in each eye, and an inter-eye difference < eight mOsm/L). The researchers report that "A possible alternate diagnosis was established in 89% of patients with normal tear osmolarity testing. The most frequent diagnoses included anterior blepharitis (26%) and allergic conjunctivitis (21%)" (Brissette et al., 2019). This highlights the utility of the TearLab test to differentiate between DED and other eye disorders with overlapping symptoms.

In a retrospective study by Tashbayev et al. (2020), 757 patients diagnosed with symptomatic DED were recruited to investigate the clinical utility of tear osmolarity measurement. The TearLab osmometer was used to measure osmolarity in both eyes and the results were compared to Ocular Surface Disease Index (OSDI), TFBUT, ocular surface staining (OSS), Schrimer test, and meibomian gland functionality tests. According to their data, TearLab results were not significantly different between the healthy controls and the DED patients. Many studies confirm that tear osmolarity greater than 308 MOsm/mL indicates a loss of homeostasis in the tear, therefore, is used as a cut-off value. Many of the healthy controls had tear osmolarity levels above the recommended cut-off value of 308 mOsm/L, and a substantial proportion of the diagnosed DED patients had tear osmolarity levels below the cut-off value. In the DED patient group, osmolarity levels in the right and left eye were 275–398 mOsm/L and 272–346 mOsm/L, respectively. In the control group, osmolarity levels in the right and left eyes were 281–369 mOsm/L and 275–398 mOsm/L, respectively. Therefore, the authors suggest that "tear osmolarity measured with TearLab osmometer cannot be used as a key indicator of DED" (Tashbayev et al., 2020).

As shown in the above studies, there have been issues in the past regarding the use of tear osmolarity as a diagnostic tool. First, no criteria for the measurement of osmolarity have been established. Studies reviewing osmolarity as a diagnostic tool do not use uniform numbers in their calculations (i.e., no uniform cut-off values, no standardized severity measures, etc.). To compound this issue, high variance in osmolarity due to outside factors, such as sleep deprivation, altitude, or even whether the right or left eye was used to produce the tears, can occur. This difficulty in establishing osmolarity ranges has caused an overlap between the ranges of healthy and dry eye osmolarity. Although measuring fluctuations between osmolarity readings has been suggested as a diagnostic (caused by increased instability), the line between healthy eyes and dry eyes is blurred (Willcox et al., 2017). However, a recent report by the TFOS DEWS II states that tear osmolarity "is a global, early stage marker of the disease and has been shown to be able to effectively track therapeutic response and inform the clinician as to whether there has been a loss of tear film homeostasis" (Craig, Nichols, et al., 2017).

MMP Enzymes

Chan et al. (2016) aimed to assess the utility of MMP-9 measurement in patients with post-laser-assisted in situ keratomileusis (LASIK) dry eyes compared to aged-matched controls. The *InflammaDry* was used to measure MMP-9 levels in tear film. Results showed that "The tear film MMP-9 levels were 52.7±32.5 ng/mL in dry eyes and 4.1±2.1 ng/mL in normal eyes ($p<0.001$). MMP-9 levels were >40 ng/mL in seven out of 14 (50.0%) post-LASIK dry eyes. The *InflammaDry* was positive in eight out of 14 (57.1%) post-LASIK eyes. All positive cases had tear film MMP-9 levels ≥38.03 ng/mL. Agreement between *InflammaDry* and MMP-9 was excellent with Cohen κ value of 0.857 in post-LASIK dry eyes" (Chan et al.,

2016). However, only half of the post-LASIK patients with dry eyes exhibited significant inflammation with heightened levels of MMP-9 (Chan et al., 2016).

A cross-sectional study by Jun JH (2020) investigated if the tear volume in dry eye disease (DED) patients affects the results of the MMP-9 immunoassay (*Inflammadry*). A total of 188 DED patients were enrolled in the study. Positive MMP-9 tests were confirmed in 120 patients, and negative results were noted in 68 patients. However, the authors observed that with a small sample volume, the reliability of the test result was impaired. The manufacturer also pointed out that less than six µl of sample volume could produce false-negative results. In this study, patients with higher tear volumes showed higher band densities, but subjects with lower tear volumes showed lower band densities on the immunoassay. In conditions such as Sjögren syndrome that present with markedly decreased tear secretion, *Inflammadry* could display negative results despite the elevated tear MMP-9 concentration. In addition, "among the participants of the present study, a strong positive band was identified even in patients with mild or nearly no fluorescein staining of the cornea and conjunctiva, who are expected to have very mild inflammatory eye surface inflammation" (Jun JH, 2020). In conclusion, this study determined the volume dependency of the MMP-9 immunoassay, which could induce false-negative results clinically (Jun JH, 2020).

Lee et al. (2021) conducted a cross-sectional study to analyze the association of MMP-9 immunoassay results with the severity of DED symptoms and signs. Using 320 patients, the researchers evaluated the clinical signs based on the OSDI score, visual analogue scale (VAS), TBUT, "tear volume evaluation by tear meniscometry, and staining scores of the cornea and conjunctiva by the Oxford grading scheme." They found that "positive MMP-9 immunoassay results were significantly related to shorter tBUT, tBUT ≤ three seconds, higher corneal staining score, corneal staining score ≥ two, and conjunctival staining score ≥ two" which indicated a worsening severity of ocular signs in DED. The researchers also performed semiquantitative analyses, basing the reagent band density on a four-point scale ranging from negative (zero) to strongly positive (three), and found that these results positively correlated with higher corneal staining scores and negatively correlated with TBUT. However, despite these correlating results, the researchers found that their quantitative analysis, which would've been the most accurate way to evaluate tear MM-9 levels, yielded no correlation between "immunoassay band density and the clinical signs and symptoms of DE." This likely indicates the need for more studies with less selection bias and greater consideration of DED subtypes, as this finding was contrary to established literature.

Choi et al. (2023) conducted a study comparing positive MMP-9 presence against an increased tear osmolarity measurement to diagnose severity of DED. The researchers found that those who tested positive for MMP-9 via immunoassay had "higher corneal fluorescein staining score and worse DED severity," as well as a worse ocular surface staining score with statistical significance. In using a cutoff for tear osmolarity level of 308 mOSm/L, they found "no significant difference in dry eye signs and symptoms," but "higher tear osmolarity was associated with ocular surface staining score in patients with severe DED and [evaporative dry eye]." Though historically tear osmolarity has been found to be useful in diagnosing DED, the researchers attribute the difference in their current findings to the study population, and that it may be better for identifying clinical severity in those already diagnosed with DED by other means (Choi et al., 2023).

Lactoferrin

A meta-analysis was performed to highlight the potential role of tear lactoferrin as a diagnostic biomarker for DED. All original studies reporting an estimate of the average lactoferrin concentration in

healthy subjects and those affected by DED were searched. A pooled mean difference of 0.62 (95% CI, 0.35–0.89) in lactoferrin concentration was observed in DED patients, showing a significant decrease in lactoferrin concentrations in the tears of subjects affected by DED. A study reported that administration of lactoferrin protein in mice led to a decrease in oxidative damage and an enhancement of tear function (Kawashima et al., 2012). Lastly, the author notes that "to compare data across studies and to validate lactoferrin as a diagnostic biomarker, there is still a need for further development of standardized protocols of tear collection, processing and storage" (Ponzini et al., 2020).

Guidelines and Recommendations

Dysfunctional Tear Syndrome (DTS) Panel

A study assessed the new diagnostic techniques and treatment options for DED and associated tear film disorders. Experts from the Cornea, External Disease, and Refractive Society (DTS Panel) convened by the study found examining tear osmolarity useful in diagnosis "in combination with other clinical assessments and procedures." The same panel also stated that the use of MMP-9 may only be valid for more severe cases of dry eye since the diagnostic test is only positive past 40 ng/mL. The panel recommended that osmolarity be evaluated before any ocular surface assessment, then an evaluation of ocular inflammation can be done, and finally a Schirmer strip test should be done (Milner et al., 2017).

American Academy of Ophthalmology (AAO)

The AAO states "no single test is adequate for establishing the diagnosis of dry eye" and recommends that the combination of findings from diagnostic tests can be useful to understanding a patient's condition. In particular, the AAO states, "tests results should be considered within the context of symptoms and other clinical findings." "Pharmacological and procedural treatments are associated with improvements in patient symptoms and clinical signs, although chronic therapy and patient compliance are necessary in most instances. Topical cyclosporine treatment has long been used in the treatment of dry eye and shown to have clinical benefits. Topical cyclosporine, in some instances, leads to long-term treatment-free remission remission of patient symptoms and signs. Lifitegrast is a lymphocyte function-associated antigen-1 antagonist developed to treat dry eye syndrome (also known as dry eye disease), but the exact mechanism of action of lifitegrast in dry eye is unknown. Topical lifitegrast five percent has been approved by the US Food and Drug Administration for treatment of dry eye" (Akpek et al., 2019). In their 2022 summary benchmarks for the cornea/external disease preferred practice pattern guidelines, the AAO cites that diagnostic tests may include "tear break-up time, ocular surface dye staining, Schirmer test, fluorescein dye disappearance test/tear function index, and tear osmolarity test" (AAO, 2022).

Rather than relying solely on a single measure of tear osmolarity, correlation with clinical findings or differences in osmolarity over time or under different conditions is more informative for confirming the diagnosis of dry eye. Indeed, most recent studies confirm that normal subjects have exceptionally stable tear film osmolarity, whereas tear osmolarity values in dry eye subjects become unstable quickly and lose homeostasis with environmental changes. These data reinforce the long-held belief that tear film instability due to increased evaporation of tears resulting in hyperosmolarity (i.e., evaporative dry eye) is a core mechanism of the disease" (Akpek et al., 2019). The guideline covers the currently used diagnostic tests, which are as follows: assessment of tear osmolarity, MMP-9, tear production, fluorescein dye or

tear function index, tear break up time, ocular surface dye staining, and lacrimal gland function (Akpek et al., 2019). The following table is provided by Akpek et al. (2019):

Table 2: Characteristic Findings for Dry Eye Disease Diagnostic Tests

Test	Characteristic Findings
Tear osmolarity	Elevated; test-to-test variability; inter-eye differences considered abnormal
Matrix metalloproteinase-9	Indicates presence of inflammation which dictates treatment
Aqueous tear production (Schirmer test)	10 mm or less considered abnormal
Fluorescein dye disappearance test/tear	Test result is compared with a standard color scale
Tear break-up time	Less than 10 seconds considered abnormal
Ocular surface dye staining	Staining of inferior cornea and bulbar conjunctiva typical
Lacrimal gland function	Decreased tear lactoferrin concentrations

Tear Film & Ocular Surface (TFOS) Society

The TFOS society held the International Dry Eye Workshop II in 2017. From this workshop, the society published recommendations on the management and treatment of DED. The authors state that when diagnosing DED, it is important to distinguish between the type (aqueous deficient dry eye or evaporative dry eye) and to determine the underlying etiology as this is crucial for proper management (Craig, Nelson, et al., 2017). These guidelines also stated that "neurotrophic keratopathy accompanied by neuropathic pain and symptoms should definitely be considered in differential diagnosis of patients with intense symptoms despite mild signs" (Craig, Nelson, et al., 2017).

Regarding diagnostic testing, the TFOS states that any patient who obtains a positive score on the Dry Eye Questionnaire-5 or Ocular Surface Disease Index should be subject to a clinical examination. "The presence of any one of three specified signs; reduced non-invasive break-up time; elevated or a large interocular disparity in osmolarity; or ocular surface staining (of the cornea, conjunctiva or lid margin) in either eye, is considered representative of disrupted homeostasis, confirming the diagnosis of DED. If a patient has DED symptoms and their practitioner does not have access to all these tests, a diagnosis is still possible, based on a positive result for any one of the markers, but may require referral for confirmation if the available homeostasis markers are negative" (Craig, Nelson, et al., 2017). After confirmation with any of the aforementioned tests (i.e. reduced non-invasive break-up time less than ten seconds, an elevated or large interocular disparity in osmolarity ≥308 m0sm/L in either eye or an interocular difference greater than eight m0sm/L, or ocular surface staining including greater than five corneal spots, greater than nine conjunctival sports, or a lid margin ≥ two mm in length and ≥ 25% in width), further evaluation should be conducted including meibography, lipid interferometry, and tear volume measurement to assess severity and help determine the best treatment plan (Craig, Nelson, et al., 2017).

Further, the consensus recommendation from the society on tear osmolarity testing states, "The low variation of normal subjects contributes to the high specificity of the marker and makes it a good candidate for parallelization and therapeutic monitoring. Accordingly, normal subjects don't display elevated osmolarity, so a value over 308 mOsm/L in either eye or a difference between eyes greater than

eight mOsm/L are good indicators of a departure from tear film homeostasis and represent a diseased ocular surface" (Craig, Nichols, et al., 2017).

Regarding MMP-9 testing, the guidelines state that "With the availability of newer immunosuppressive medications and trials concerning these drugs it is logical that inflammation should be assessed. The exact modality used may need to be varied depending on the pathway or target cell upon which the immunosuppressive drug acts, and such diagnostic tools should be used for refining patient selection as well as monitoring after commencement of treatment. Costs of these diagnostic tests should be considered, but these should be calculated from a holistic standpoint. For example, if the tests can assist the channeling of patients to appropriate healthcare services there may be cost savings for reduced referrals" (Craig, Nichols, et al., 2017).

American Optometric Association

The AOA published consensus-based clinical practice guidelines for care of a patient with ocular surface disorders. These guidelines note that there is a "lack of a defined diagnostic test or protocol and a lack of congruity between patient symptoms and clinical tests." The AOA also notes that the condition itself is ill defined and that dry eye is often a symptom of another condition such as blepharitis or another glandular dysfunction (AOA, 2010). There have not been any updates on this topic from the AOA since this 2010 statement.

Consensus Guidelines for Management of Dry Eye Associated with Sjögren Disease

In 2015, clinical guidelines for management of dry eye associated with Sjögren disease were published by a consensus panel which evaluated reported treatments for DED. The recommendations state, "Evaluation should include symptoms of both discomfort and visual disturbance as well as determination of the relative contribution of aqueous production deficiency and evaporative loss of tear volume. Objective parameters of tear film stability, tear osmolarity, degree of lid margin disease, and ocular surface damage should be used to stage severity of dry eye disease to assist in selecting appropriate treatment options. Patient education with regard to the nature of the problem, aggravating factors, and goals of treatment is critical to successful management. Tear supplementation and stabilization, control of inflammation of the lacrimal glands and ocular surface, and possible stimulation of tear production are treatment options that are used according to the character and severity of dry eye disease" (Foulks et al., 2015). Further, tear osmolarity was identified as the testing method with the highest level of evidence for all DED related tests.

American Society of Cataract and Refractive Surgery (ASCRS) Cornea Clinical Committee

American Society of Cataract and Refractive Surgery (ASCRS) released guidelines to aid surgeons in diagnosing visually significant ocular surface disorders (OSD) before refractive surgery. The ASCRS Cornea Clinical Committee recommends initial screening procedures including ASCRS Standard Patient Evaluation of Eye Dryness (SPEED) II questionnaire, tear osmolarity, and matrix metalloproteinase (MMP-9) testing. If any of the three initial screening tests are abnormal, the patient is at risk for ocular surface disease, and additional diagnostic tests can be performed to determine dry eye sub-type. Non-invasive tests are recommended to minimize disruption to the ocular surface, cornea, and tear film. These tests include tear lipid layer thickness, noninvasive tear breakup time (NIKBUT), tear meniscus height, meibography, topography, tear lactoferrin levels, and measures of optical scatter. However, these tests are not essential to the fundamental algorithm.

The ASCRS also notes a point of care test that assesses lactoferrin levels (TearScan). The guideline notes its three proprietary biomarkers which are as follows: "salivary protein-1 (SP-1, immunoglobulin A [IgA], immunoglobulin G [IgG], immunoglobulin M [IgM]); (2) carbonic anhydrase-6 (CA-6, IgA, IgG, IgM); and (3) parotid secretory protein (PSP, IgA, IgG, IgM)". The authors comment that this test can be used to detect Sjögren syndrome early. However, the authors also note that "no member of the ASCRS Cornea Clinical Committee has used it [TearScan] in clinical practice" (Starr et al., 2019).

Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

On December 3, 1993, the FDA approved the lactoferrin microassay system by Touch Scientific, Inc (FDA, 2024). Lactoferrin diagnostic kits are commercially available options for tear film biomarkers (Willcox et al., 2017).

On May 14, 2009, the FDA approved TearLab created by Ocusense Inc. From the FDA site: this device is used "to measure the osmolality of human tears to aid in the diagnosis of patients with signs or symptoms of DED, in conjunction with other methods of clinical evaluation" (TearLab, 2023).

On November 20, 2013, the FDA approved InflammaDry created by Rapid Pathogen Screening Inc. From the FDA site: "InflammaDry is a rapid, immunoassay test for the visual, qualitative in vitro detection of elevated levels of the MMP-9 protein in human tears from patients suspected of having dry eye to aid in the diagnosis of dry eye in conjunction with other methods of clinical evaluation. This test is intended for prescription use at point-of-care sites" (FDA, 2013).

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). As an LDT, the U. S. Food and Drug Administration has not approved or cleared this test; however, FDA clearance or approval is not currently required for clinical use.

Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
82785	Gammaglobulin (immunoglobulin); IgE
83516	Immunoassay for analyte other than infectious agent antibody or infectious agent antigen; qualitative or semiquantitative, multiple step method
83520	Immunoassay for analyte other than infectious agent antibody or infectious agent antigen; quantitative, not otherwise specified
83861	Microfluidic analysis utilizing an integrated collection and analysis device, tear osmolarity

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

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Revision History

Revision	Summary of Changes
03/06/2024	Reviewed and Updated: Updated the background, guidelines and recommendations, and evidence-based scientific references. Literature review did not necessitate any modifications to coverage criteria.
03/01/2023	Reviewed and Updated: Updated the background, guidelines and recommendations, and evidence-based scientific references. Literature review did not necessitate any modifications to coverage criteria. The following edits were made for clarity: All CC edited for clarity and consistency. For clarity, CC1 broken into subcriteria: "1) For individuals suspected of having dry eye, testing of tear osmolarity MEETS COVERAGE CRITERIA in any of the following situations: a) To help determine the severity of dry eye disease. b) To monitor effectiveness of therapy."
03/09/2022	Annual review: Updated background, guidelines and recommendations, and evidence-based scientific references. Literature review did not necessitate modification to coverage criteria.
03/03/2021	Annual review: Updated background, guidelines and recommendations, and evidence-based scientific references. Literature review did not necessitate modification to coverage criteria.
09/08/2020	Off-cycle review: Updated background, guidelines, and evidence-based scientific references. Literature review necessitated the following changes to the CC: CC1 was switched from DNMCC to MCC based on the 2017 TFOS International Dry Eye Workshop II. The CC now states: 1. Testing of tear osmolarity in patients suspected of having dry eye **MEETS COVERAGE CRITERIA** to aid in determining the severity of dry eye disease as well as monitor effectiveness of therapy.

	2. Testing for MMP-9 protein in human tears **DOES NOT MEET COVERAGE CRITERIA** to aid in the diagnosis of patients suspected of having dry eye disease based on comprehensive eye examination.
03/10/2020	Annual review: Updated background, guidelines and recommendations, and evidence-based scientific references. Added in statement regarding lack of published literature (for the old E&I CC) and changed the old E&I CC to DNMCC. Literature review did not necessitate any other modifications to the CCs.
03/01/2019	Annual review: Updated the background, federal regulations, guidelines, and evidence-based scientific references. Added two new CCs: Lactoferrin and/or IgE testing to aid in diagnosis of DED DNMCC All other testing to aid in diagnosis of DED is E&I Added CPT 83516, 83520 & 83861.
03/16/2018	Off-Cycle Review: Policy was reviewed to change the Annual Review Cycle. Literature review did not necessitate any modification to coverage criteria. No changes in coding.
09/15/2017	Annual review: Definitions, Background, Guidelines and Recommendations and Evidence-based Scientific References were updated. Literature review did necessitate change in CC: added coverage criteria CC2 for tear osmolarity testing.
09/19/2016	Initial presentation

Fecal Analysis in the Diagnosis of Intestinal Dysbiosis and Fecal Microbiota Transplant Testing

Policy Number: AHS – G2060 – Fecal Analysis in the Diagnosis of Intestinal Dysbiosis and Fecal Microbiota Transplant Testing	Prior Policy Name and Number, as applicable: • AHS – G2060 – Fecal Analysis in the Diagnosis of Intestinal Dysbiosis
Initial Presentation Date: 09/18/2015 Revision Date: February 1, 2025	

Policy Description

Intestinal dysbiosis is defined as a disruption or imbalance of the intestinal microbial ecology (Guinane & Cotter, 2013). Dysbiosis is associated with many diseases, including irritable bowel syndrome (IBS), inflammatory bowel diseases (IBD), celiac disease, multiple sclerosis, Sjogren's Syndrome, obesity, allergy, and diabetes (Carding et al., 2015; Marietta et al., 2020).

Related Policies

Policy Number	Policy Title
AHS-G2056	Diagnosis of Idiopathic Environmental Intolerance
AHS-G2061	Fecal Calprotectin Testing
AHS-G2121	Laboratory Testing for the Diagnosis of Inflammatory Bowel Disease

Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) Prior to fecal microbiota transplant (FMT), fecal analysis by culture for the following microorganisms **MEETS COVERAGE CRITERIA:**
    a) Extended spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae*
    b) Vancomycin-resistant *Enterococci* (VRE)
    c) Carbapenem-resistant *Enterobacteriaceae* (CRE)
    d) Methicillin-resistant *Staphylococcus aureus* (MRSA)
    e) *Campylobacter*
    f) *Shigella*
    g) *Salmonella*

2) Prior to fecal microbiota transplant (FMT), fecal analysis for the following microorganisms by nucleic acid amplification testing (NAAT) **MEETS COVERAGE CRITERIA:**
    a) *Clostridium difficile*
    b) *Campylobacter*
    c) *Salmonella*
    d) *Shigella*
    e) Shiga toxin-producing *Escherichia coli*
    f) Norovirus
    g) Rotavirus
    h) COVID-19 (SARS-CoV-2)

3) Prior to fecal microbiota transplant (FMT), fecal analysis for the following microorganisms by nucleic acid amplification testing (NAAT) **DOES NOT MEET COVERAGE CRITERIA**:
    a) Extended spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae*
    b) Vancomycin-resistant *Enterococci* (VRE)
    c) Carbapenem-resistant *Enterobacteriaceae* (CRE)
    d) Methicillin-resistant *Staphylococcus aureus* (MRSA)
    e) Any other microorganisms not listed above

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

4) As a diagnostic test for the evaluation of intestinal dysbiosis, irritable bowel syndrome, malabsorption, **or** small intestinal overgrowth of bacteria, fecal analysis of the following components **DOES NOT MEET COVERAGE CRITERIA**:

   a) Triglycerides

   b) Chymotrypsin

   c) Iso-butyrate, iso-valerate, and n-valerate

   d) Meat and vegetable fibers

   e) Long chain fatty acids

   f) Cholesterol

   g) Total short chain fatty acids

   h) The levels of *Lactobacilli*, bifidobacteria, and *E. coli* and other "potential pathogens," including *Aeromona*, *Bacillus cereus*, *Campylobacter*, *Citrobacter*, *Klebsiella*, *Proteus*, *Pseudomonas*, *Salmonella*, *Shigella*, *S. aureus*, *Vibrio*

   i) For the identification and quantitation of fecal yeast (including *C. albicans*, *C. tropicalis*, *Rhodoptorul* and *Geotrichum*)

   j) N-butyrate

   k) Beta-glucoronidase

   l) pH

   m) Short chain fatty acid distribution (adequate amount and proportions of the different short chain fatty acids reflect the basic status of intestinal metabolism)

   n) Fecal secretory IgA

Table of Terminology

Term	Definition
ACG	American College of Gastroenterology
AGA	American Gastroenterological Association
ASGE	American Society for Gastrointestinal Endoscopy
BSG	British Society of Gastroenterology
CBC	Complete blood cell count
CD	Crohn's disease
CDI	Clostridium difficile infection
CMS	Centers for Medicare and Medicaid Services
CPE	Carbapenemase-producing Enterobacteriaceae/Enterobacterales
CRE	Carbapenem-resistant Enterobacteriaceae/Enterobacterales
CRP	C-reactive protein
ECCO	European Crohn’s and Colitis Organization
EIA	Enzyme immunoassay
ELISA	Enzyme-linked immunoassay

EMA	Endomysial antibodies
EPEC	Enteropathogenic *Escherichia coli*
ESBL	Extended spectrum beta-lactamase
ESGAR	European Society of Gastrointestinal and Abdominal Radiology
ESPGHAN	European Society for Pediatric Gastroenterology, Hepatology, and Nutrition
ESPID	European Society for Pediatric Infectious Diseases
ESR	Erythrocyte sedimentation rate
FBC	Full blood count
FDA	Food and Drug Administration
FGFP	Flemish gut flora project
FMT	Fecal microbiota transplant
GI	Gastrointestinal
HIS	Healthcare Infection Society
IBD	Inflammatory bowel disease
IBS	Irritable bowel syndrome
IDSA	Infectious Diseases Society of America
IMO	Intestinal methanogenic overgrowth
IND	Investigational new drug
MDROs	Multidrug resistant organisms
MRSA	Methicillin-resistant staphylococcus aureus
NAAT	Nucleic acid amplification testing
NASPGHAN	North American Society for Pediatric Gastroenterology, Hepatology and Nutrition
NICE	National Institute for Health and Care Excellence
SATs	Single-arm trials
SBBO	Small bowel bacterial overgrowth
SCFA	Short-chain fatty acids
SIBO	Small intestinal bacterial overgrowth
STEC	Shigatoxin-producing *Escherichia coli*
TTG	Tissue transglutaminase
UC	Ulcerative colitis
VRE	Vancomycin-resistant enterococci
WGO	World Gastroenterology Organization

Scientific Background

The human intestinal tract has a diverse and complex microbial community necessary for health and nutrition. The gut microbiome is estimated to consist of upwards of 1000 bacterial species (Guinane & Cotter, 2013; Ley, Peterson, et al., 2006; Qin et al., 2010). The microbiota functions with the immune system to protect against pathogens. It also performs essential metabolic functions, extracting certain forms of energy and nutrients from food and providing a source of other essential nutrients and vitamins (Carding et al., 2015).

The gut is colonized at birth, but the intestinal microbiome changes rapidly during the first year of life. In adults, each individual's unique population of gut microbiota is fairly stable over time; however, alterations in the microbiota can result from exposure to various environmental factors, including diet, toxins, drugs, and pathogens (Carding et al., 2015; Lozupone et al., 2012; Snapper & Abraham, 2022). This change in an individual's normal microbiota is called "dysbiosis" (Johnston Jr, 2023). Dysbiosis has been associated with obesity (Ley, Turnbaugh, et al., 2006; Zhang et al., 2009) malnutrition (Kau et al., 2011), systematic diseases such as diabetes (Qin et al., 2012) and chronic inflammatory diseases such as inflammatory bowel disease (IBD) (Frank et al., 2007; Guinane & Cotter, 2013). Both direct assessment of the gut microbiota (examination of bacteria levels) and indirect assessment (measurement of non-living markers such as pH or beta-glucoronidase) have been proposed for investigation of intestinal dysbiosis.

Microbial or microbial-derived components have also been cited as potential representations of dysbiosis. For example, short-chain fatty acids have been identified as a mechanism to regulate intestinal processes and, as such, may represent dysbiosis (Johnston Jr, 2023). These fatty acids are the products of bacterial fermentation of fiber, and the concentrations of these fatty acids have been noted to decrease in IBD cases. Some fatty acids, especially butyrate, have been demonstrated to factor in signaling cascades that control immune function, which indicates a role in controlling intestinal inflammation (Parada Venegas et al., 2019). Ongoing research has uncovered many other potential links between intestinal metabolism and gut microbiota so many markers have been suggested as potential indicators of dysbiosis.

Many tests exist for the assessment of the gut microbiome. Due to the amount of conditions associated (or proposed to be associated) with gut microbiome balance, there are many corresponding tests, including screening measures intended for completely healthy individuals. These tests primarily revolve around nucleic acid amplification; microbial DNA or RNA is obtained from the sample, unique sequences are identified, and the nucleic acid is quantified (Raby, 2020). For instance, Viome offers a comprehensive screening panel that measures "all microorganisms" in the gut (including viruses, archaea, yeast, fungi, parasites, and bacteriophages). Those measurements are combined into a score for various issues, such as inflammatory activity, digestive efficiency, methane gas production, overall gas production, and more (Viome, 2023). Viome also provides a list of nutritional recommendations, broken down into individual foods. Viome performs RNA sequencing with Illumina NextSeq and uses bioinformatics algorithms to classify taxonomic data (Viome, 2019).

Some companies may offer companion products with their gut microbiome tests. BioHM provides a similar assessment of bacterial and fungal species in an individual's gastrointestinal tract, but the company also offers a series of probiotics. These probiotics are intended for various purposes, such as colon cleansing or immunity (BioHM, 2023). Other companies offering a gut microbiome test include Thryve, GenCove, DayTwo, American Gut, and Genova (DNATestingChoice, 2019; Genova, 2023).

The potential clinical impact of imbalance in the intestinal microbiota suggests a need for standardized diagnostic methods to facilitate microbiome profiling. Documenting dysbiosis has traditionally relied on classical microbiological techniques and the ability to culture pure isolates for identification and classification; however, the ability to classify bacteria and archaea according to individual 16S rRNA sequences can now possibly provide a rapid and detailed means of profiling complex communities of microorganisms (Casen et al., 2015; Zoetendal et al., 1998). Laboratory analysis of various fecal biomarkers have also been proposed as a method of identifying individuals with intestinal dysbiosis and may be useful in providing insight into the role of intestinal health and disease, and the development of non-gastrointestinal conditions associated with intestinal dysbiosis. However, there is a current lack of

literature on the normal ranges of these biomarkers, which limit the applicability of these analyses in a general clinical setting (Bäckhed et al., 2012; Berry & Reinisch, 2013; Pang et al., 2014).

A technique revolving around restoring balance in a patient's microbiome is fecal microbiota transplantation (FMT). FMT is the infusion of stool from a healthy donor to a patient with presumed gut dysbiosis. The concept behind this technique is that the healthy donor's stool can facilitate a restoration of the ill patient's gut microbiome. This technique has seen some significant success in the treatment of *C. difficile* infections and may have potential applications in some other gastrointestinal or metabolic conditions such as IBD or IBS. As with any transplant procedure, there are several screening procedures that must be undertaken to minimize risk of infection or other disease transmission. These screening procedures include evaluation of donor history, serum testing, and stool testing. The pathogens screened for in the donor's stool sample may vary between institutions, although some pathogens are universally screened for (such as enteric pathogens) (Kim & Gluck, 2019).

Clinical Utility and Validity

Falony et al. (2016) analyzed "two independent, extensively phenotyped cohorts: the Belgian Flemish Gut Flora Project (FGFP; discovery cohort; N = 1106) and the Dutch LifeLines-DEEP study (LLDeep; replication; N = 1135)." These two sets were integrated with global data sets, combining to yield 3948 items. A "core" set of 14 genera was identified. 69 clinical and questionnaire-based covariates were found to be associated with microbiota compositional variation with a 92% replication rate. The authors noted that "stool consistency showed the largest effect size, whereas medication explained largest total variance and interacted with other covariate-microbiota associations, but early-life events such as birth mode were not reflected in adult microbiota composition" (Falony et al., 2016).

Zhernakova et al. (2016) sequenced the gut microbiomes of 1,135 participants from a Dutch population-based cohort. The authors identified relations between the microbiome and "126 exogenous and intrinsic host factors, including 31 intrinsic factors, 12 diseases, 19 drug groups, 4 smoking categories, and 60 dietary factors." "Significant" associations were found between the gut microbiome and various intrinsic, environmental, dietary, medication parameters, and disease phenotypes. The authors calculated that 18.7% of variation in microbial composition could be explained by these factors, and they observed that fecal chromogranin A was exclusively associated with 61 microbial species, totaling 53% of the microbial composition. A more diverse microbiome was associated with low CgA concentrations. The authors concluded that "these results are an important step toward a better understanding of environment-diet-microbe-host interactions" (Zhernakova et al., 2016).

Lo Presti et al. (2019) profiled the fecal and mucosal microbiota of IBD and IBS patients. 38 IBD patients, 44 IBS patients, and 47 healthy controls were included, and overall, 107 fecal samples were provided. The authors found that "*Anaerostipes* and *Ruminococcaceae* were identified as the most differentially abundant bacterial taxa in controls, *Erysipelotrichi* was identified as [a] potential biomarker for IBS, while *Gammaproteobacteria*, *Enterococcus*, and *Enterococcaceae* [were identified] for IBD" (Lo Presti et al., 2019).

Malham et al. (2019) investigated the microbiotic profile of pediatric IBD. 143 IBD patients and 34 healthy controls were included. A reduced "richness" in microbiotic profile was observed in IBD patients compared to healthy controls. In ulcerative colitis (UC), that reduced richness was associated with high intestinal inflammation and extensive disease. Nine species were "significantly" associated with a healthy microbiome, and three species were associated with IBD. The authors remarked that the microbiome

composition could differentiate between Crohn's Disease, UC, and healthy controls (Malham et al., 2019).

Danilova et al. (2019) compared the gut microbiome composition of IBD patients to healthy controls. 95 IBD patients and 96 healthy controls were included. The authors noted an increase of Proteobacteria and Bacteroidetes bacteria and decrease of Firmicutes bacteria and Euryarchaeota archaea in IBD patients. Butyrate-producing and hydrogen-utilizing bacteria were observed to have lower representation in IBD patients. Short-chain fatty acids (SCFA) were also found to have a lower absolute content in IBD patients. The authors suggested that this finding may "indicate inhibition of functional activity and number of anaerobic microflora and/or an [*sic*] change in SCFA utilization by colonocytes" (Danilova et al., 2019).

Vaughn et al. (2018) in reviewing the current status of intestinal dysbiosis and fecal transplantation found that "it is hypothesized that intestinal dysbiosis may contribute to the pathogenesis of many diseases, especially those involving the gastrointestinal tract. Therefore, fecal microbiota transplantation (FMT) is increasingly being explored as a potential treatment that aims to optimize microbiota composition and functionality" (Vaughn et al., 2018). Holleran et al. (2018) also found that fecal transplant is not recommended for use outside of *Clostridium difficile* infection (CDI) due to concerns regarding outcome and safety; however, several case series and randomized controlled trials have described its use in a research environment for a few gastrointestinal conditions related to intestinal dysbiosis, including ulcerative colitis (UC), Crohn's disease (CD) and irritable bowel syndrome (IBS). The most successful reports of the clinical efficacy of FMT in gastrointestinal conditions outside of CDI have been in treating UC (Holleran et al., 2018).

Costello et al. (2019) evaluated fecal microbiota transplantation (FMT)'s efficacy on inducing remission in ulcerative colitis (UC). The authors compared anaerobically prepared donor FMT (n = 38) to autologous FMT (stool provided by patient themselves, n = 35). The primary outcome was defined as "steroid-free remission of UC… a total Mayo score of ≤2 with an endoscopic Mayo score of 1 or less at week 8." A total of 69 patients completed the trial, with the primary outcome being achieved in 12 of 38 donor FMT patients, compared to 3 of 35 receiving autologous FMT. Five of the 12 patients achieving the primary outcome in the "donor cohort" maintained remission at 12 months. The authors concluded that "in this preliminary study of adults with mild to moderate UC, 1-week treatment with anaerobically prepared donor FMT compared with autologous FMT resulted in a higher likelihood of remission at 8 weeks. Further research is needed to assess longer-term maintenance of remission and safety" (Costello et al., 2019).

Myneedu et al. (2019) performed a meta-analysis to evaluate whether fecal microbiota transplantation (FMT) was successful in treating IBS. A total of 8 single-arm trials (SATs, 90 patients total) and 5 randomized controlled trials (RCTs, 151 patients, 105 controls) were included. In the SAT cohort, the authors identified 59.5% of IBS patients demonstrating a significant improvement. In the RCT cohort, there were no significant differences between treatment and control cohorts, either by the IBS Severity Scoring System or the IBS Quality of Life (IBS-QOL). The authors concluded that "FMT was not effective in IBS. Variations in FMT methods and patient factors may contribute to the heterogeneous results of the trials" (Myneedu et al., 2019).

In a prospective survey-based study, Saha et al. (2021) studied the long-term safety profile of fecal microbiota transplantation (FMT) for recurrent C. *difficile* infection (CDI). 609 patients who underwent FMT were contacted at 1 week, 1 month, 6 months, 1 year and greater than 2 years after transplantation. Symptoms and new medical diagnosis were recorded at each time point. Less than 1 year after FMT, greater than 60% of patients had diarrhea and 19-33% had constipation. At 1 year, 9.5% of patients

reported additional CDI episodes. Additionally, patients with IBD, dialysis dependent kidney disease, and multiple FMTs had a higher risk of diarrhea. When patients were followed up after 2 years post-FMT, 73 new diagnoses were reported including gastrointestinal disorders (13%), weight gain (10%), and new infections unrelated to FMT (11.8%). The median time for new infections post-FMT was 29 months. The authors conclude that FMT "appears safe with low risk of transmission of infections. Several new diagnoses were reported, which should be explored in future studies" (Saha et al., 2021).

In a 12-week double-blind placebo-controlled pilot trial, Yu et al. (2020) studied the use of FMT to improve metabolic outcomes in obese patients. From a total of 24 patients, 12 adults with obesity and mild to moderate insulin resistance were given weekly oral FMT capsules from healthy lean donors and 12 adults were given. At 0, 6, and 12 weeks, various metabolic parameters were measured including HbA1c, body weight, body composition, and resting energy expenditure. According to the results, there were no significant differences between the two groups in glycemic outcomes, weight, or body composition over the 12-week period. There was a minor improvement in HbA1c after FMT as compared to placebo. These results suggest "that intestinal microbial manipulation by FMT capsules does not meaningfully alter human metabolism and weight in adults with obesity" (Yu et al., 2020).

Macareño-Castro et al. (2022) conducted a systematic review on the use of FMT on Carbapenem-resistant Enterobacteriaceae. In using 10 studies with a combination of both retrospective and prospective cohorts, they found that among 112 FMT recipients with confirmed CRE, 78.7% of patients experienced CRE decolonization at the end of study follow-up (6-12 months). The predominant strains reported were *Klebsiella pneumoniae* and *Escherichia coli*. The researchers also reported that there were no “severe complications even in immunosuppressed patients and in those with multiple underlying conditions.” This overall supports the clinical utility of FMT for CRE, but requires more studies, such as randomized trials, to validate the safety and reliable use for complete bacterial eradication.

Guidelines and Recommendations

World Gastroenterology Organization (WGO) Global Guidelines

The WGO published guidelines on functional gastrointestinal (GI) symptoms. In it, they identify diagnostic tests for these symptoms. The basic diagnostic tests are as follows:

- Complete blood cell count (CBC)
- Erythrocyte sedimentation rate (ESR) / C-reactive protein (CRP)
- Biochemistry panel
- Fecal occult blood (patient aged > 50 y)
- Pregnancy test
- Liver function tests
- Calprotectin or other fecal test to detect inflammatory bowel disease in patients thought to have IBS, but in whom inflammatory bowel disease (IBD) is a possibility; now routine in many primary care settings (in the United Kingdom)
- Celiac serology; considered routine in areas with a high prevalence of celiac disease
- Stool testing for ova and parasites (Hunt et al., 2014)

The WGO also released their global guidelines for Inflammatory Bowel Disease in 2015 (published in 2016). Their recommendations concerning stool examination and testing are as follows:

- "Routine fecal examinations and cultures should be carried out to eliminate bacterial, viral, or parasitic causes of diarrhea."
- "Testing for *Clostridium difficile* (should be considered even in the absence of antecedent antibiotics) — should be carried out within 2 hours of passage of stools."
- "A check for occult blood or fecal leukocytes should be carried out if a patient presents without a history of blood in the stool, as this can strengthen the indication for lower endoscopy. Where lower endoscopy is readily available, these tests are rarely indicated."
- "Lactoferrin, α1-antitrypsin. The main reason for listing this test is to rule out intestinal inflammation, rather than using it as a positive diagnostic test. It may not be available in developing countries, but it can be undertaken relatively inexpensively and easily with rapid-turnaround slide-based enzyme-linked immunoassay (ELISA) tests."
- "Calprotectin — a simple, reliable, and readily available test for measuring IBD activity — may be better for UC than CD; the rapid fecal calprotectin tests could be very helpful in developing countries. If available, a home test may be useful as a routine for follow-up" (Bernstein et al., 2016).

American Gastroenterological Association (AGA)

The AGA published a review to "describe key principles in the diagnosis and management of functional gastrointestinal (GI) symptoms in patients with inflammatory bowel disease". In it, they include the following relevant items:

- "Alternative pathophysiologic mechanisms should be considered and evaluated (small intestinal bacterial overgrowth, bile acid diarrhea, carbohydrate intolerance, chronic pancreatitis) based on predominant symptom patterns."
- "Until further evidence is available, fecal microbiota transplant should not be offered for treatment of functional GI symptoms in IBD."
- "In a recent cross-sectional analysis, no association was observed between IBS symptoms and microbiome alterations among patients with IBD although effects of confounding could not be excluded" (Colombel et al., 2019).

The AGA published guidelines on FMT, including information on donor pathogen screening. *C. difficile* toxin B and culture for enteric pathogens were "suggested" to be screened for, *Giardia*, *Cryptosporidium*, *Isospora* and *Cyclospora*, *Listeria*, *E. coli* O157, *Vibrio*, and *Norovirus* should be "considered", and Cytomegalovirus, Human T-cell lymphoma virus, Epstein–Barr virus, *Dientamoeba fragilis*, *Blastocystis hominis*, *Strongyloides stercoralis*, *Entamoeba histolytica*, *H. pylori*, *Schistosoma*, JC virus, Vancomycin-resistant *enterococci*, and Methicillin-resistant *Staphylococcus aureus* should "maybe" [term used by authors] be screened (Kelly et al., 2015).

American College of Gastroenterology (ACG)

The ACG published a guideline regarding the management of Crohn's Disease. In it, they recommend that "In patients who have symptoms of active Crohn's disease, stool testing should be performed to include fecal pathogens, *Clostridium difficile* testing, and may include studies that identify gut inflammation such as a fecal calprotectin" (Lichtenstein et al., 2018).

The ACG also published a guideline regarding management of ulcerative colitis. In it, the ACG writes that "FMT requires more study and clarification of treatment before use as a therapy for UC [ulcerative

colitis]." The ACG comments that the variability across all steps of the procedure (donor screening, delivery, treatment duration, et al.) makes interpretation of the current results "difficult". Finally, the ACG notes that some institutions have been using "comprehensive intestinal pathogen testing through PCR-based assays that include many bacterial and viral pathogens," but that the "prevalence and impact of non–C. diff intestinal pathogens detected through such assays remain to be robustly established" (Rubin et al., 2019).

ACG published a guideline regarding management of irritable bowel syndrome. ACG does not recommend the use of fecal transplant for the treatment of global IBS symptoms. "Evidence to support FMT for the treatment of IBS is limited and of very low quality and thus cannot be recommended at present" (Lacy et al., 2021).

ACG published a guideline regarding use of FMT in recurrent and severe C. *difficile* infection. ACG suggests considering FMT for "patients with severe and fulminant CDI refractory to antibiotic therapy, in particular, when patients are deemed poor surgical candidates. For patients experiencing their second or further recurrence of CDI, FMT can be delivered to prevent further recurrences through capsule or colonoscopy. Enema may be used if other methods are unavailable." ACG suggests "repeat FMT for patients experiencing a recurrence of CDI within 8 weeks of an initial FMT. FMT should be considered for recurrent CDI in patients with IBD" (Kelly et al., 2021).

European Crohn's and Colitis Organization (ECCO) and the European Society of Gastrointestinal and Abdominal Radiology (ESGAR)

These joint guidelines include some relevant items on inflammatory bowel disease (IBD), which includes both Crohn's disease (CD) and ulcerative colitis (UC). These items include:

- "At diagnosis, every patient should have a biochemical assessment with full blood count, inflammatory markers (C-reactive protein [CRP])… and a stool sample for microbiological analysis, including *C. difficile*."
- "Stool specimens should be obtained to exclude common pathogens and specifically assayed for C difficile toxin." (Maaser et al., 2019)

2012 Rome Foundation Report

An international Working Group convened in 2012 "to provide clinical guidance on modulation of gut microbiota in IBS" and released their findings on intestinal microbiota in functional bowel disorders: a Rome foundation report in 2013. They state the following "Diagnostic and therapeutic general recommendations":

- "There is currently no clinically useful way of identifying whether the microbiota are disturbed in particular patients with irritable bowel syndrome (IBS).
- Dietary evaluation and exclusion of possible sources of unabsorbable carbohydrates including fermentable oligo-, di- and mono-saccharides and polyols and excessive fibre could be beneficial in select patients.
- Probiotics have a reasonable evidence base and should be tried, for a period of at least 1 month, at adequate doses before a judgement is made about the response to treatment.
- The utility of testing for small intestinal bacterial overgrowth (SIBO) in the setting of IBS remains an area of uncertainty.

- If SIBO is strongly suspected based on clinical presentation and testing is being considered, using stringent criteria for the glucose breath test or jejunal aspirate appear to be the best tests.
- Consideration should be given to discontinuing proton pump inhibitors in those with SIBO.
- There is emerging evidence that non-absorbable antibiotics may have the potential to reduce symptoms in some patients with IBS" (Simren et al., 2013).

European Society for Pediatric Gastroenterology, Hepatology, and Nutrition/European Society for Pediatric Infectious Diseases (ESPGHAN/ESPID)

These joint guidelines reviewed management of acute gastroenteritis (AGE) in children. In it, they note that AGE does not require a specific diagnostic workup and that "microbiological investigation is not helpful in most cases." Fecal markers are also not recommended for differentiating viral and bacterial AGE. However, the guidelines observe that "microbiological investigations may be considered in children with underlying chronic conditions (eg, oncologic diseases, IBDs, etc), in those in extremely severe conditions, or in those with prolonged symptoms in whom specific treatment is considered" (Guarino et al., 2014).

National Institute for Health and Care Excellence (NICE)

NICE updated their IBS guidelines in 2017. In it, they list the following items about diagnostic tests:

"In people who meet the IBS diagnostic criteria, the following tests should be undertaken to exclude other diagnoses:

- full blood count (FBC)
- erythrocyte sedimentation rate (ESR) or plasma viscosity
- c-reactive protein (CRP)
- antibody testing for coeliac disease (endomysial antibodies [EMA] or tissue transglutaminase [TTG]).

The following tests are not necessary to confirm diagnosis in people who meet the IBS diagnostic criteria:

- ultrasound
- rigid/flexible sigmoidoscopy
- colonoscopy; barium enema
- thyroid function test
- faecal ova and parasite test
- faecal occult blood
- hydrogen breath test (for lactose intolerance and bacterial overgrowth)" (NICE, 2017).

British Society of Gastroenterology (BSG)

The BSG published a guideline on the investigation of chronic diarrhoea in adults. Relevant items include:

- For malabsorption, fecal tests have not received "significant support" in publications and have not "established themselves in clinical practice outside specialist centres."
- "We suggest culture of small bowel aspirates as it is the most sensitive test for small bowel bacterial overgrowth (SBBO), but methods are poorly standardized and positive results may not

reflect clinically significant SBBO… in the absence of an optimal test to confirm the presence of bacterial overgrowth and in those with a high test probability of SBBO, we recommend an empirical trial of antibiotics; the value of this approach has not been subject to definitive study."
- "We recommend faecal elastase testing as the preferred non-invasive test for pancreatic function" (Arasaradnam et al., 2018).

The BSG also published an extensive guideline on the management of Inflammatory Bowel Disease (including both ulcerative colitis (UC) and Crohn's disease) in adults. Their relevant comments and recommendations include:

- "In patients presenting with suspected UC, stool cultures and *Clostridium difficile* toxin assay should always be performed to rule out infective causes."
- "Ileocolonoscopy with biopsy is established as the first-line investigation for suspected Crohn's disease."
- "We recommend that all patients presenting with acute flares of colitis should have stool cultures for enteroinvasive bacterial infections and stool *Clostridium difficile* assay."
- "In spite of these encouraging data, FMT [Faecal microbial transplantation] remains an investigational treatment for use only in clinical trials in IBD."
- "There is currently no place for FMT in the management of IBD unless complicated by *C. difficile* infection outside of the clinical trial setting" (Lamb et al., 2021).

British Society of Gastroenterology (BSG) and Healthcare Infection Society (HIS)

This joint guideline was published to provide guidance on "the use of faecal microbiota transplant as treatment for recurrent or refractory *Clostridium difficile* infection and other potential indications." These guidelines include a list of items that should be screened for potential stool donors, which are as follows:

- "*Clostridium difficile* PCR"
- "*Campylobacter*, *Salmonella*, and *Shigella* by standard stool culture and/ or PCR"
- "Shiga toxin-producing *Escherichia coli* by PCR"
- "Multi-drug resistant bacteria, at least CPE [*carbapenemase-producing Enterobacteriaceae*] and ESBL [extended spectrum beta-lactamase]"
- "Stool ova, cysts and parasite analysis, including for Microsporidia"
- "Faecal antigen for *Cryptosporidium* and *Giardia"*
- "Acid fast stain for *Cyclospora* and *Isospora"*
- *"Helicobacter pylori* faecal antigen"
- "Norovirus, rotavirus PCR."

The above list is for stool screening. A separate list is provided for serum screening. The guideline also recommends that "donors should have successfully completed a donor health questionnaire and laboratory screening assays both before and after the period of stool donation" (Mullish et al., 2018).

Infectious Diseases Society of America/American College of Gastroenterology/American Society for Gastrointestinal Endoscopy/American Gastroenterological Association/North American Society for Pediatric Gastroenterology, Hepatology and Nutrition (IDSA/ACG/ASGE/AGA/NASPGHAN)

These joint guidelines were sent to the FDA regarding recurrent *Clostridium difficile* infection (CDI). In it, the guidelines recommend screening donors for fecal microbiota transplantation (FMT) for *C. difficile* toxin B and performing a culture for enteric pathogens (IDSA/ACG/ASGE/AGA/NASPGHAN, 2013).

NASPGHAN published an FMT guideline for children in 2019, and the same analytes for screening (C difficile toxin B, culture for enteric pathogens) were recommended (Davidovics et al., 2019).

An addendum was published to the 2019 guidelines due to the 2019 FDA Safety Warning regarding FMT. In it, the following recommendation was made: "FMT donor stool screening should include (but not be limited to) MDRO testing for spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae*, vancomycin-resistant *Enterococci* (VRE), carbapenem-resistant *Enterobacteriaceae* (CRE), and methicillin-resistant *Staphylococcus aureus* (MRSA). Donors and/or stools positive for MDROs should not be used for FMT" (Michail et al., 2020).

Food and Drug Administration (FDA)

The FDA has issued a guidance statement for fecal microbiota transplant (FMT) stating that it will exercise enforcement discretion regarding the investigational new drug (IND) requirements for the use of fecal microbiota for transplantation. In 2019, the FDA updated their guidance on FMT, stating that "FMT donor stool testing must include MDRO testing to exclude use of stool that tests positive for MDRO. The MDRO tests should at minimum include extended spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae, vancomycin-resistant enterococci (VRE), carbapenem-resistant Enterobacteriaceae (CRE), and methicillin-resistant Staphylococcus aureus (MRSA). Culture of nasal or peri-rectal swabs is an acceptable alternative to stool testing for MRSA only. Bookend testing (no more than 60 days apart) before and after multiple stool donations is acceptable if stool samples are quarantined until the post-donation MDRO tests are confirmed negative" (FDA, 2019).

In an April 2020 update, the FDA addressed the topic of fecal microbiota transplantation within the context of the 2020 COVID-19 outbreak. The FDA included additional protections regarding stool donation and donor screening, which are as follows:

- "Stool donor screening, including an assessment of whether, since December 1, 2019, the donor was diagnosed with laboratory-confirmed SARS-CoV-2 infection, experienced symptoms of COVID-19 (e.g., fever, cough, shortness of breath) not explained by another diagnosis, or was exposed to a suspected or confirmed case of COVID-19 or SARS-CoV-2 infection."
- "Testing of the stool donation or stool donor for SARS-CoV-2 virus or RNA. Testing approaches might include testing upper respiratory specimens (e.g., nasal swabs) or other specimens (e.g., rectal swabs or stool donations)" (FDA, 2020a).

In a March 2020 update, the FDA addressed the potential risk of infections with the use of FMT. The FDA advises that "patients considering FMT for the treatment of *C. difficile* infection should speak to their health care provider to understand the associated risks" (FDA, 2020b). The FDA is aware of infections caused by enteropathogenic *Escherichia coli* (EPEC) and *Shigatoxin-producing Escherichia coli* (STEC) that have occurred following investigational use of FMT (FDA, 2020b).

Fecal Microbiota Transplantation Workgroup (2011)

This Working Group published guidelines on FMT. Fecal donor screening recommendations were included. The following analytes were recommended to be screened:

- "*C difficile* toxin B by PCR; if unavailable, then evaluation for toxins A and B by enzyme immunoassay (EIA)
- Routine bacterial culture for enteric pathogens
- Fecal Giardia antigen

- Fecal Cryptosporidium antigen
- Acid-fast stain for Cyclospora, Isospora, and, if antigen testing unavailable, Cryptosporidium
- Ova and parasites
- *Helicobacter pylori* fecal antigen (for upper gastrointestinal [GI] routes of FMT administration)" (Bakken et al., 2011).

Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
82239	Bile acids; total
82542	Column chromatography, includes mass spectrometry, if performed (eg, HPLC, LC, LC/MS, LC/MS-MS, GC, GC/MS-MS, GC/MS, HPLC/MS), non-drug analyte(s) not elsewhere specified, qualitative or quantitative, each specimen
82705	Fat or lipids, feces; qualitative
82710	Fat or lipids, feces; quantitative
82715	Fat differential, feces, quantitative
82725	Fatty acids, nonesterified
82784	Gammaglobulin (immunoglobulin); IgA, IgD, IgG, IgM, each
83520	Immunoassay for analyte other than infectious agent antibody or infectious agent antigen; quantitative, not otherwise specified
83630	Lactoferrin, fecal; qualitative
83986	pH; body fluid, not otherwise specified
84311	Spectrophotometry, analyte not elsewhere specified
87045	Culture, bacterial; stool, aerobic, with isolation and preliminary examination (eg, KIA, LIA), Salmonella and Shigella species
87046	Culture, bacterial; stool, aerobic, additional pathogens, isolation and presumptive identification of isolates, each plate
87075	Culture, bacterial; any source, except blood, anaerobic with isolation and presumptive identification of isolates
87102	Culture, fungi (mold or yeast) isolation, with presumptive identification of isolates; other source (except blood)

CPT	Code Description
87177	Ova and parasites, direct smears, concentration and identification
87209	Smear, primary source with interpretation; complex special stain (eg, trichrome, iron hemotoxylin) for ova and parasites
87328	Infectious agent antigen detection by immunoassay technique, (eg, enzyme immunoassay [EIA], enzyme-linked immunosorbent assay [ELISA], fluorescence immunoassay [FIA], immunochemiluminometric assay [IMCA]) qualitative or semiquantitative; cryptosporidium
87329	Infectious agent antigen detection by immunoassay technique, (eg, enzyme immunoassay [EIA], enzyme-linked immunosorbent assay [ELISA], fluorescence immunoassay [FIA], immunochemiluminometric assay [IMCA]) qualitative or semiquantitative; giardia
87336	Infectious agent antigen detection by immunoassay technique, (eg, enzyme immunoassay [EIA], enzyme-linked immunosorbent assay [ELISA], fluorescence immunoassay [FIA], immunochemiluminometric assay [IMCA]) qualitative or semiquantitative; Entamoeba histolytica dispar group
87493	Infectious agent detection by nucleic acid (DNA or RNA); Clostridium difficile, toxin gene(s), amplified probe technique
87500	Infectious agent detection by nucleic acid (DNA or RNA); vancomycin resistance (eg, enterococcus species van A, van B), amplified probe technique
87641	Infectious agent detection by nucleic acid (DNA or RNA); Staphylococcus aureus, methicillin resistant, amplified probe technique
87798	Infectious agent detection by nucleic acid (DNA or RNA), not otherwise specified; amplified probe technique, each organism
89160	Meat fibers, feces
S3708	Gastrointestinal fat absorption study

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

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Revision History

Revision Date	Summary of Changes
09/06/2023	Reviewed and Updated: Updated the background, guidelines and recommendations, and evidence-based scientific references. Literature review did not necessitate any modifications to coverage criteria. The following edits were made for clarity: Addition of ":" at the end of the main body of CC1 and CC2.

Fecal Calprotectin Testing in Adults

Policy Number: AHS – G2061 – Fecal Calprotectin Testing in Adults	Prior Policy Name and Number, as applicable: AHS – G2061 – Fecal Calprotectin Testing
Initial Presentation Date: 09/18/2015 Revision Date: February 1, 2025	

Policy Description

Calprotectin is a small calcium-binding protein found in high concentration in the cytosol of neutrophils (Fagerhol et al., 1980) and to a lesser extent monocytes and macrophages (Hsu et al., 2009). Active intestinal inflammation and disturbance of the mucosa results in entrance of neutrophils (containing calprotectin) into the lumen and subsequent excretion in feces. Detection of fecal calprotectin is used to distinguish inflammatory bowel disease (IBD) from irritable bowel syndrome (IBS) and other causes of abdominal discomfort, bloating, and diarrhea (Walsham & Sherwood, 2016).

Related Policies

Policy Number	Policy Title
AHS-G2060	Fecal Analysis in the Diagnosis of Intestinal Dysbiosis and Fecal Microbiota
AHS-G2121	Laboratory Testing for the Diagnosis of Inflammatory Bowel Disease
AHS-G2155	General Inflammation Testing

Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) For individuals 18 years of age or older, fecal calprotectin testing for the differential diagnosis between non-inflammatory gastrointestinal disease (e.g., IBS) and inflammatory gastrointestinal disease (e.g., IBD) **MEETS COVERAGE CRITERIA**.

2) For individuals 18 years of age or older, fecal calprotectin testing either to assess for response to therapy or for relapse or to monitor gastrointestinal conditions such as inflammatory bowel disease (IBD) **MEETS COVERAGE CRITERIA**.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

3) For individuals 18 years of age or older, fecal calprotectin testing for all other situations not discussed above **DOES NOT MEET COVERAGE CRITERIA**.

Table of Terminology

Term	Definition
ACG	American College of Gastroenterology
AGA	American Gastrointestinal Association
CD	Crohn's disease
CDAI	Crohn's disease activity index
C. diff	*Clostridioides difficile*
CI	Confidence interval
CRP	C-reactive protein
DOR	Diagnostic odds ratio
ECCO	European Crohn's and Colitis Organisation
ELISA	Enzyme linked immunosorbent assay
ESGAR	European Society of Gastrointestinal and Abdominal Radiology
ESR	Erythrocyte sedimentation rate
FC	Fecal calprotectin
FCAL	Fecal calprotectin
FDA	Food and Drug Administration
FL	Fecal lactoferrin
GI	Gastrointestinal
IBD	Inflammatory bowel disease
IBS	Irritable bowel syndrome
NICE	National Institute for Health and Care Excellence
SAM	Severe acute malnutrition
SES-CD	Simple endoscopic score for Crohn disease
SROC	Summary receiver operating characteristic
TNFα	Tumor necrosis factor alpha
UC	Ulcerative colitis

Scientific Background

Inflammatory bowel disease (IBD) includes several chronic, immune-mediated inflammatory gastrointestinal disorders, the most common being Crohn's disease and ulcerative colitis (Boirivant & Cossu, 2012). In contrast, irritable bowel syndrome (IBS), another gastrointestinal disorder, is a non-inflammatory condition. These disorders often share similar symptoms including abdominal discomfort, pain, bloating, and diarrhea (Burri & Beglinger, 2014). An estimated two thirds of Americans have experienced these IBS and/or IBD symptoms (Almario et al., 2018). Differentiating gastrointestinal tract symptoms due to IBS from those due to residual inflammation from IBD is challenging (Gibson, 2022; Halpin & Ford, 2012). However, the detection of fecal calprotectin can be used to effectively distinguish between these conditions (Walsham & Sherwood, 2016).

Calprotectin is a small calcium- and zinc-binding protein. This protein is primarily detected in monocytes and macrophages. During active intestinal inflammation, neutrophils migrate to the mucosa, damaging the mucosal structure. This causes leakage of these neutrophils and therefore calprotectin into the lumen and eventually the feces. Calprotectin is homogenously distributed in feces, is stable up to seven days at room temperature, and correlates well with the "gold standard" of the indium-labeled leukocyte test (Walsham & Sherwood, 2016).

Fecal calprotectin is now accepted as one of the most useful tools to assist with the clinical management of IBD, although the optimal cut-off laboratory value for both differentiating IBD from IBS and managing IBD may vary depending on clinical settings (Khaki-Khatibi et al., 2020; Maaser et al., 2019; Mumolo et al., 2018). A value of 50 µg/g is quoted by most manufacturers of calprotectin kits (Tibble et al., 2002). In a young patient, a cutoff of 150 µg/g is recommended. As fecal calprotectin is increased in gastroenteritis associated with viral or bacterial infection, a value between 50 µg/g and 150 µg/g should always be repeated two to three weeks later (Walsham & Sherwood, 2016).

Fecal calprotectin is typically measured with polyclonal or monoclonal antibodies that detect various features on the protein structure; these tests may be quantitative or qualitive. Manufacturers of this type of test include Calpro and Bühlmann (Walsham & Sherwood, 2016).

Clinical Utility and Validity

Fecal calprotectin is increasing in utilization for the evaluation of IBD (Higuchi & Bousvaros, 2022). Meta-analyses of fecal calprotectin by both von Roon et al. (2007) and van Rheenen et al. (2010) found an overall sensitivity and specificity for IBD of >90%. Waugh et al. (2013) also completed a meta-analysis as part of the national Health Technology Assessment program which found a pooled sensitivity of 93% and specificity of 94% when distinguishing between IBS and IBD in adults with a fecal calprotectin cut-off of 50 µg/g.

Molander et al. (2012) evaluated fecal calprotectin levels after induction therapy with TNFα antagonists to determine whether this treatment can help to predict the outcome of IBD patients during maintenance therapy. Sixty patients with IBD were treated with TNFα antagonists and had their fecal calprotectin measured. Fecal calprotectin was found to be normalized (≤100 µg/g) in 31 patients and elevated in 29 patients. After 12 months, 26 of the 31 patients with normal fecal calprotectin levels were in clinical remission whereas only 11 of the 29 with elevated fecal calprotectin were in remission. A cutoff concentration of 139 µg/g was found to have a sensitivity of 72% *and* specificity of 80% to predict a risk of clinically active disease after one year (Molander et al., 2012).

Molander et al. (2015) studied whether fecal calprotectin can predict relapse after stopping TNFα-blocking therapy in IBD patients in remission. Forty-nine patients were examined, of which 15 relapsed (34 in remission). Relapsing patients showed an elevated fecal calprotectin for a median of 94 days before relapsing. Normal fecal calprotectin levels were "highly predictive" of clinical and endoscopic remission. The authors suggested that fecal calprotectin may be used as "a surrogate marker for predicting and identifying patients requiring close follow-up in clinical practice" (Molander et al., 2015).

Mao et al. (2012) performed a meta-analysis of the predictive capacity of fecal calprotectin in IBD relapse. A total of 672 patients (318 with ulcerative colitis, 354 with Crohn's Disease) from six studies were examined. The authors found the pooled sensitivity and specificity of fecal calprotectin to predict relapse of quiescent IBD to be 78 and 73%, respectively. The area under the summary receiver-operating characteristic (sROC) curve was 0.83, and the diagnostic odds ratio was 10.31. The authors concluded that "as a simple and noninvasive marker, FC [fecal calprotectin] is useful to predict relapse in quiescent IBD patients" (Mao et al., 2012).

Rosenfeld et al. (2016) published a study to evaluate the perspective of gastroenterologists regarding the impact of fecal calprotectin on the management of patients with IBD. A total of 279 completed surveys were collected. Ninety surveys indicated fecal calprotectin testing was used to differentiate IBD from IBS, 85 indicated that fecal calprotectin was used to differentiate IBS symptoms from IBD in IBD patients, and 104 indicated fecal calprotectin was used as a marker for objective inflammation. Fecal calprotectin levels also resulted in a management change in 143 surveys, including 118 fewer colonoscopies. Overall, 272 surveys stated they would order fecal calprotectin again (Rosenfeld et al., 2016).

Abej et al. (2016) investigated the association between fecal calprotectin and other measures of clinical activity for patients with IBD. A total of 240 patients with IBD contributed 183 fecal samples, and a fecal calprotectin measurement above ≥250 µg was considered a positive result. Fecal calprotectin was associated with "colonoscopy findings of active IBD, low albumin, anemia, and elevated CRP." The authors concluded that fecal calprotectin "is a useful marker of disease activity and a valuable tool in managing persons with IBD in clinical practice" (Abej et al., 2016).

Tham et al. (2018) showed that fecal calprotectin is an accurate surrogate marker of postoperative endoscopic recurrence of Crohn's disease. They evaluated the diagnostic sensitivity, specificity, and diagnostic odds ratio (DOR), and constructed summary receiver operating characteristic (SROC) curves in a meta-analysis of 54 studies; Nine studies were eligible for analysis. Diagnostic accuracy was calculated for fecal calprotectin values of 50, 100, 150 and 200 µg/g. A significant threshold effect was observed for all fecal calprotectin values. The optimal diagnostic accuracy was obtained for a fecal calprotectin value of 150 µg/g, with a pooled sensitivity of 70% [95% confidence interval (CI) 59-81%], specificity 69% (95% CI 61-77%), and DOR 5.92 (95% CI 2.61-12.17); the area under the SROC curve was 0.73 (Tham et al., 2018).

The cost-effectiveness of the use of fecal calprotectin in the diagnosis of IBD has been investigated (Yang et al., 2014). The authors compared cost-effectiveness of measuring fecal calprotectin before endoscopy compared to direct endoscopic evaluation alone. Fecal calprotectin screening was found to save $417 per adult patient, but delayed 2.2/32 adult diagnoses (of IBD. The authors noted that if endoscopic biopsy remained the diagnostic standard, direct endoscopic evaluation would cost an additional $18955 in adults to avoid one false-negative result from fecal calprotectin screening (Yang et al., 2014).

In a cross-sectional study, Campbell et al. (2021)assessed the clinical performance of the LIAISON Calprotectin Assay in differentiating inflammatory bowel disease (IBD) from irritable bowel syndrome (IBS) against the Genova Diagnostics PhiCal test. A total of 240 patients were included in the study in which 102 patients had IBD, 67 had IBS, and 71 had other GI disorders. Median fecal calprotectin levels were higher in IBD patients (522 µg/g) compared to IBS patients (34.5 µg/g). The LIAISON assay showed good correlation with the PhiCal test, holding a positive percent agreement of 97.8% and a negative percent agreement of 94.4%. Overall, the LIAISON Calprotectin Assay is efficient with a time to the first result of 35 minutes and "is a sensitive marker for distinguishing IBD from IBS with a cutoff of ~100 µg/g" (Campbell et al., 2021).

Johnson et al. (2022) compared fecal calprotectin and pancreatic elastase assays, aiming to understand the differences between the tests and manufacturers. Data from proficiency tests performed in Germany between 2015 and 2020 was included in the study. Fecal calprotectin assays had a "high degree of variability" between tests from the eight manufactures included. Pancreatic elastase assays were "harmonized" without significant variability between tests from the five manufacturers included. The authors concluded that "both calprotectin and pancreatic elastase assays could be improved by standardization efforts" (Johnson et al., 2022).

Guidelines and Recommendations

National Institute for Health and Care Excellence (NICE)

The NICE published guidance on fecal calprotectin testing which included the following recommendations:

- "Fecal calprotectin testing is recommended as an option to support clinicians with the differential diagnosis of inflammatory bowel disease (IBD) or irritable bowel syndrome (IBS) in adults with recent onset lower gastrointestinal symptoms for whom specialist assessment is being considered, if cancer is not suspected and appropriate quality assurance processes and locally agreed care pathways are in place for the testing" (NICE, 2017).

American Gastrointestinal Association (AGA)

The AGA published a practice update on functional gastrointestinal symptoms in patients with IBD. The following best practice advice recommendations on fecal calprotectin were given regarding the diagnosis and management of functional gastrointestinal symptoms in patients IBD:

- "Best practice advice 1: A stepwise approach to rule-out ongoing inflammatory activity should be followed in IBD patients with persistent GI symptoms (measurement of fecal calprotectin, endoscopy with biopsy, cross-sectional imaging).
- Best practice advice 2: In those patients with indeterminate fecal calprotectin levels and mild symptoms, clinicians may consider serial calprotectin monitoring to facilitate anticipatory management" (Colombel et al., 2019).

In 2023, the AGA published guidelines on the role of biomarkers for management of ulcerative colitis (Singh et al., 2023). For patients with ulcerative colitis in symptomatic remission, the AGA recommends that:

- "In patients with UC in symptomatic remission, the AGA suggests a monitoring strategy that combines biomarkers and symptoms, rather than symptoms alone."

- "In patients with UC in symptomatic remission, the AGA suggests using fecal calprotectin <150 µg/g, normal fecal lactoferrin, or normal CRP to rule out active inflammation and avoid routine endoscopic assessment of disease activity."
- "In patients with UC in symptomatic remission, the AGA suggests using fecal calprotectin <150 µg/g, normal fecal lactoferrin, or normal CRP to rule out active inflammation and avoid routine endoscopic assessment of disease activity."

For patients with symptomatically active ulcerative colitis, the AGA recommends that:

- "In patients with symptomatically active UC, the AGA suggests an evaluation strategy that combines biomarkers and symptoms, rather than symptoms alone, to inform treatment adjustments."
- "In patients with symptomatically active UC, the AGA suggests an evaluation strategy that combines biomarkers and symptoms, rather than symptoms alone, to inform treatment adjustments."
- "In patients with UC with mild symptoms, with elevated stool or serum markers of inflammation (fecal calprotectin >150 µg/g, elevated fecal lactoferrin, or elevated CRP), the AGA suggests endoscopic assessment of disease activity rather than empiric treatment adjustment."
- "In patients with UC with mild symptoms, with normal stool or serum markers of inflammation (fecal calprotectin <150 µg/g, normal fecal lactoferrin, normal CRP), the AGA suggests endoscopic assessment of disease activity rather than empiric treatment adjustment."

For treat-to-target strategies for ulcerative colitis, the AGA recommends that:

- "In patients with UC, the AGA makes no recommendation in favor of, or against, a biomarker-based monitoring strategy over an endoscopy-based monitoring strategy to improve long-term outcomes" (Singh et al., 2023).

American College of Gastroenterology (ACG)

The ACG Clinical Guideline (Lichtenstein et al., 2018) for the Management of Crohn's disease in adults recommends:

"Fecal calprotectin is a helpful test that should be considered to help differentiate the presence of IBD from irritable bowel syndrome (IBS) (strong recommendation, moderate level of evidence)."

"In patients who have symptoms of active Crohn's disease, stool testing should be performed to include fecal pathogens, *Clostridium difficile* testing, and may include studies that identify gut inflammation such as a fecal calprotectin."

"Fecal calprotectin and fecal lactoferrin measurements may have an adjunctive role in monitoring disease activity. Fecal markers may have a role in noninvasively monitoring disease activity in CD [Crohn's disease]. Studies have shown that both fecal lactoferrin and fecal calprotectin are sensitive markers of disease activity and correlate with a number of the endoscopic activity indices such as the colonic SES-CD. There have been several studies that suggest that levels of fecal calprotectin can be used to monitor patients for postoperative recurrence after ileocolic resection for Crohn's disease. Levels of >100 µ g/g indicate endoscopic recurrence with a sensitivity in the range of 89%. In patients with an infliximab-induced remission, fecal calprotectin of >160 µ g/g has a sensitivity of 91.7% and a specificity of 82.9% to predict relapse… The presence of biomarkers of disease activity can be assessed (such as CRP, fecal calprotectin) but should not exclusively serve as end point for treatment as

normalization of the biomarker can occur despite having active mucosal inflammation/ulceration… Although not specific for CD activity, determination of serum CRP and/or fecal calprotectin is suggested as a useful laboratory correlate with disease activity assessed by the CDAI" (Lichtenstein et al., 2018).

The Crohn's Disease Activity Index (CDAI) is a tool that can provide a numerical value in assessing Crohn's disease; however, fecal calprotectin is not a criterion of the index. Within the supplemental information of the guidelines, the authors state, "This is a weighted subjective tool that includes scores for liquid bowel movements per day, general wellbeing, abdominal pain and extra-intestinal manifestations. This index does require 7 days of measurements making it difficult to use in the clinic setting. Due to the subjective nature of some of the measurements it is not an optimal tool for measuring disease activity and is generally not used in routine clinical practice"(Lichtenstein et al., 2018).

The guidelines do not address the frequency of fecal calprotectin testing for adjunctive monitoring.

The ACG also published guidelines for clinical management of ulcerative colitis in adults in 2019. In it, they note that "Fecal calprotectin (FC) can be used in patients with UC as a noninvasive marker of disease activity and to assess response to therapy and relapse" (Rubin et al., 2019). The ACG also recommends:

- "Stool testing to rule out *Clostridioides difficile (C. diff)* in patients suspected of having UC (strong recommendation, very low quality of evidence)."
- Recommends against "serologic antibody testing to establish or rule out a diagnosis of UC (strong recommendation, very low quality of evidence)."
- Recommends against serologic antibody testing to determine the prognosis of UC (strong recommendation, very low quality of evidence)" (Rubin et al., 2019).

In 2021, the ACG published guidelines on the management of irritable bowel syndrome. They recommend that that fecal calprotectin, either fecal calprotectin 1 or fecal lactoferrin 2 and C-reactive protein 1, be checked in patients with suspected IBS and diarrhea symptoms to rule out inflammatory bowel disease. ACG includes that two fecal-derived markers of intestinal inflammation, fecal lactoferrin (FL) and fecal calprotectin (fCal), are both diagnostically useful and could be superior to serologic tests such as CRP or ESR regarding discriminating IBD from IBS. "In summary, fCal and FL are safe, noninvasive, generally available, and can identify IBD with good accuracy" (Lacy et al., 2021).

European Crohn's and Colitis Organisation (ECCO)

The ECCO released a consensus on diagnosis and management of ulcerative colitis (UC). In it, they state that fecal calprotectin should be included on an initial investigation of UC. ECCO considers fecal calprotectin an "accurate" marker of colonic inflammation and "a useful non-invasive marker in the follow-up of UC patients" (Magro et al., 2017).

The ECCO also provided a statement on diagnosis and management of Crohn's Disease. ECCO notes that fecal calprotectin may be used in the initial laboratory investigation. Fecal calprotectin is also observed to be an emerging surrogate marker for mucosal healing but has not demonstrated a clear predictive value. Fecal calprotectin may also help in monitoring disease activity (Gomollón et al., 2016).

European Crohn's and Colitis Organisation (ECCO) and the European Society of Gastrointestinal and Abdominal Radiology (ESGAR)

The ECCO-ESGAR published guidelines for the diagnostic assessment in IBD. When monitoring known IBD cases, the following guidelines were provided:

- "Response to treatment in active ulcerative colitis [UC] should be determined by a combination of clinical parameters, endoscopy, and laboratory markers such as C-reactive protein [CRP] and faecal calprotectin [EL1]
- In patients with UC who clinically respond to medical therapy, mucosal healing [MH] should be determined endoscopically or by faecal calprotectin [FC] approximately 3 to 6 months after treatment initiation [EL5]" (Maaser et al., 2019).

A relevant portion of "**Table 1.** Markers of disease activity for monitoring asymptomatic IBD patients" is shown below

	Validity [correlation with gold standard]	**Responsiveness to changes in condition**	**Signal-to-noise ratio [ability to differentiate changes in condition from background variability]**	**Practicality**
Endoscopy	Gold standard	Gold standard	Gold standard	Low
Faecal calprotectin	Good	Good Rises quickly in case of relapse; falls rapidly with successful treatment	Moderate Risk of false-positive results	High Possible reluctance of patients for repeated stool collection

(Maaser et al., 2019):

Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

In March 2006, the PhiCal™ (Genova Diagnostics) quantitative ELISA test for measuring concentrations of fecal calprotectin in fecal stool was cleared for marketing by the U.S. Food and Drug Administration (FDA) through the 510(k) processes. This test is indicated to aid in the diagnosis of inflammatory bowel disease (IBD) and to differentiate IBD from irritable bowel syndrome (IBS); it is intended to be used in conjunction with other diagnostic testing and clinical considerations (FDA, 2006). On December 26,

2018, a successor device called "LIAISON Calprotectin, LIAISON Calprotectin Control Set, LIAISON Calprotectin Calibration Verifiers, LIAISON Q.S.E.T. Buffer, LIAISON Q.S.E.T. Device" was approved. The new description is as follows: "The DiaSorin LIAISON® Calprotectin assay is an in vitro diagnostic chemiluminescent immunoassay (CLIA) intended for the quantitative measurement, in human stool, of fecal calprotectin, a neutrophilic protein that is a marker of mucosal inflammation. The LIAISON® Calprotectin assay can be used as an aid in the diagnosis of inflammatory bowel diseases (IBD), specifically Crohn's disease and ulcerative colitis, and as an aid in differentiation of IBD from irritable bowel syndrome (IBS). Test results are to be used in conjunction with information obtained from the patients' clinical evaluation and other diagnostic procedures. The test has to be performed on the LIAISON® XL Analyzer" (FDA, 2018a).

In January 2014, CalPrest® (Eurospital SpA, Trieste, Italy) was cleared for marketing by the FDA through the 510(k) processes. According to the FDA summary, CalPrest® "is identical" to the PhiCal™ test "in that they are manufactured by Eurospital S.p.A. Trieste, Italy. The only differences are the name of the test on the labels, the number of calibrators in the kit and the dynamic range of the assay." CalPrest®NG (Eurospital SpA) was cleared for marketing in November 2016 (FDA, 2016).

On October 16, 2018, the FDA approved the QUANTA Flash Calprotectin And Fecal Extraction Device. The device's intended use is as follows: "QUANTA Flash Calprotectin is a chemiluminescent immunoassay for the quantitative determination of fecal calprotectin in extracted human stool samples. Elevated levels of fecal calprotectin, in conjunction with clinical findings and other laboratory tests, can aid in the diagnosis of inflammatory bowel disease (IBD) (ulcerative colitis and Crohn's disease), and in the differentiation of IBD from irritable bowel syndrome (IBS)." This device has a predicate device, which was approved in 2017 (FDA, 2018a).

On December 26, 2018, the FDA approved the LIAISON Calprotectin Assay. The device's intended use is as follows: "The DiaSorin LIAISON® Calprotectin assay is an in vitro diagnostic chemiluminescent immunoassay (CLIA) intended for the quantitative measurement, in human stool, of fecal calprotectin, a neutrophilic protein that is a marker of mucosal inflammation. The LIAISON® Calprotectin assay can be used as an aid in the diagnosis of inflammatory bowel diseases (IBD), specifically Crohn's disease and ulcerative colitis, and as an aid in differentiation of IBD from irritable bowel syndrome (IBS). Test results are to be used in conjunction with information obtained from the patients' clinical evaluation and other diagnostic procedures" (FDA, 2018b).

On September 24, 2019, BUHLMANN Laboratories AG received FDA approval for the Buhlmann FCAL Turbo And CALEX Cap fecal calprotectin extraction device. This device is to be used in conjunction with the automated calprotectin test, BÜHLMANN fCAL® turbo. The BÜHLMANN fCAL® turbo is an in vitro diagnostic assay which quantitatively measures fecal calprotectin (FDA, 2019).

Rapid fecal calprotectin tests, such as CalproSmart™, are available internationally for use as point-of-care testing, but these have not been approved for use in the U.S. by the FDA.

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
83993	Assay for calprotectin fecal

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

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Revision History

Revision Date	Summary of Changes
09/06/2023	Reviewed and Updated: Updated the background, guidelines and recommendations, and evidence-based scientific references. Literature review did not necessitate any modifications to coverage criteria. The following edits were made for clarity and consistency: All CC edited to include "for all individuals 18 years of age or older" language so that it's clear that coverage in this policy is only relevant to adult individuals. Previously only clarified by "in Adults" within the title.

Flow Cytometry

Policy Number: AHS – F2019 – Flow Cytometry	Prior Policy Name and Number, as applicable:
Initial Presentation Date: 11/16/2015 Effective Date: February 1, 2025	

Policy Description

Flow cytometry is a technique for live cell analysis that measures optical light scattering features to determine physical characteristics (Adan et al., 2017). This instrument is beneficial for calculating the number of cells in a biologic sample, as well as for measuring cellular properties, such as size, shape, viability, and granularity (Verbsky & Routes, 2023). Flow cytometry may also be used for diagnostic and prognostic purposes when monitoring certain diseases, and for identifying the presence of specific biomarkers.

Flow cytometry-derived DNA content can be used for cell cycle analysis to estimate the percentages of a cell population in the various phases of the cell cycle; it can also be used with other reagents to analyze only the S phase. An S-phase fraction (SPF) is an assessment of how many cells are actively synthesizing DNA (UIHC, 2016). It is used as a measure of cell proliferation, particularly for cancer (Pinto et al., 1999). A high SPF value is indicative of rapid cancer growth (ACS, 2021).

For guidance on flow cytometry in minimal residual disease (MRD), please see AHS-M2175-Minimal Residual Disease (MRD).

Related Policies

Policy Number	Policy Title

AHS-M2175	Minimal Residual Disease
AHS-M2182	Genomic Testing for Hematopoietic Neoplasms

Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) Flow cytometry immunophenotyping of cell surface markers **MEETS COVERAGE CRITERIA** for any of the following conditions:
   a) For individuals with cytopenias, lymphomas, leukemia, myeloproliferative and lymphoproliferative disorders, or myelodysplastic syndrome.
   b) For B-cell monitoring for immunosuppressive disorders.
   c) For T-cell monitoring for HIV infection and AIDS.
   d) For individuals with mast cell neoplasms.
   e) For individuals with paroxysmal nocturnal hemoglobinuria.
   f) For preoperative or post-operative monitoring of individuals who will undergo or who have undergone organ transplantation.
   g) For individuals with plasma cell disorders.
   h) For individuals with primary immunodeficiencies (PIDs), and PIDs involving T, NK.
   i) For individuals with primary platelet disorders (non-neoplastic).
   j) For individuals with red cell and white cell disorders (non-neoplastic).

2) The following reimbursement limitations will apply for flow cytometry:
   a) For flow cytometric immunophenotyping for the assessment of potential hematolymphoid neoplasia, use codes 88184-88189.
   b) Code 88184 should be used for the first marker, per specimen, and is reimbursable up to a maximum of two units per date of service.
   c) Code 88185 should be used for each additional marker and is reimbursable up to a maximum of 35 units, per date of service.
   d) In patients with a neoplasm with an established immunophenotype, subsequent tests for that neoplasm should be limited to diagnostically relevant markers.
   e) Codes 88187, 88188, and 88189 should not be used together for a single specimen in any combination.
   f) Codes 88187, 88188, and 88189 are reimbursed at one unit per specimen, up to two specimens, per date of service.
   g) Codes 88187-88189 should not be used in conjunction with codes 86355, 86356, 86357, 86359, 86360, 86361, 86367.

h) Use codes 86355, 86357, 86359, 86360, 86361, or 86367 for cell enumeration. These codes are reimbursable as single units only.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

3) Measurement of flow cytometry-derived DNA content (DNA Index) or cell proliferative activity (S-phase fraction or % S-phase) for prognostic or therapeutic purposes in the routine clinical management of cancers **DOES NOT MEET COVERAGE CRITERIA**.

Table of Terminology

Term	Definition
AIDS	Acquired immune deficiency syndrome
AML	Acute myeloid leukemia
AMR	Antibody mediated rejection
ANKL	Aggressive NK-Cell leukemia
ASCO	American Society of Clinical Oncology
ASH	American Society of Hematology
B-ALL	B-cell precursor acute lymphoblastic leukemia
CAEBV	Chronic active Epstein-Barr virus
CAP	College of American Pathologists
CLIA '88	Clinical Laboratory Improvement Amendments of 1988
CLL	Chronic lymphocytic leukemia
CMPD	Chronic myeloproliferative disorders
CMS	Centers for Medicare and Medicaid
CR	Complete remission
CSF	Cerebrospinal fluid
DNA	Deoxyribose nucleic acid
ENKTL	Extranodal NK/T lymphoma
EBV	Epstein-Barr virus
ERIC	European Research Initiative on CLL
ESCCA	European Society for Clinical Cell Analysis
FCI	Flow cytometric immunophenotyping
FCM	Flow cytometry
FDA	Food and Drug Administration
FISH	Fluorescent in situ hybridization
FISHIS	Fluorescent in situ hybridization in suspension
FNAC	Fine needle aspiration cytology
GIST	Gastrointestinal stromal tumors
HIV	Human immunodeficiency virus infection
HSCT	Hematopoietic stem cell transplantation

IHC	Immunohistochemistry
ISGyP	International Society of Gynecological Pathologists
ISTH	International Society on Thrombosis and Haemostasis
LCDs	Local coverage determinations
LDTs	Laboratory developed tests
MDS	Myelodysplastic syndromes
MFC	Multiparameter (multicolor) flow cytometry
MRD	Minimal residual disease
NCDs	National coverage determinations
NCCN	National Comprehensive Cancer Network
NK	Natural killer
PIDs	Primary immunodeficiencies
RCUD	Refractory anemia subtype
RNA	Ribonucleic acid
RT-qPCR	Real-time quantitative polymerase chain reaction
SPF	S-phase fraction
T-ALL	T-cell acute lymphoblastic leukemia

Scientific Background

Flow cytometry is a laboratory technique with the capability to measure optical and fluorescence characteristics from single cells or other particles between 0.2 and 150 micrometers in size, such as microorganisms, nuclei or chromosome preparations suspended in fluid (Brown & Wittwer, 2000; Verbsky & Routes, 2023). More than 100 companies constitute the flow cytometry market, leading to an industry worth of more than $3 billion (Robinson & Roederer, 2015).

A typical flow cytometer contains five main components: a flow cell, a laser, optical parts, detectors which amplify signals, and an electronic or computer system (Verbsky & Routes, 2023). This device measures thousands of cells instantaneously by passing them through the laser beam, and it can even sort the cells into 96- or 384-well plates, tubes, and slides based on identified cellular properties (McKinnon, 2018). Size is determined by the forward angle light scatter, and internal properties such as cellular granularity are measured by the right-angle light scatter (Brown & Wittwer, 2000; Verbsky & Routes, 2023). These fluorescent light signals are converted into electronic signals and then analyzed by a computer to generate final results (McKinnon, 2018).

Fluorescent reagents may be used to enhance a sample before administration into the flow cytometer. These reagents may include DNA binding dyes, fluorescently conjugated antibodies, viability dyes, fluorescent expression proteins, and ion indicator dyes (McKinnon, 2018). Each fluorescent dye binds to cellular components differently, leading to distinguished outcomes when passed by the light source. A fluorochrome, or chemical that can re-emit light when excited, can assist in the detection of specific cellular properties. The use of multiple fluorochromes at once allows several characteristics to be identified instantaneously as different colors emit different wavelengths of light; common dyes include propidium iodide, phycoerythrin, and fluorescein (Brown & Wittwer, 2000).

Immunophenotyping is the most common use of flow cytometry and entails the identification of cellular markers from the immune system, such as T cell subsets and cytokines, as well as antigen-specific

responses. Unfortunately, immunophenotyping faces issues in the clinical world due to a lack of standardized procedures (Finak et al., 2016). Current instruments allow for up to 28 colors to be used in immunophenotyping experiments, yet many researchers use less than this (McKinnon, 2018).

In the field of organ transplantation, the role of flow cytometry in pre-transplant crossmatching, as well for monitoring immune reconstitution following hematopoietic stem cell transplantation, is well-established.

More recently, the utility of flow cytometry in the post-transplant setting has been recognized. Post-transplant applications of flow cytometry include antibody mediated rejection (AMR) diagnosis, graft prognosis, and therapeutic monitoring (Maguire et al., 2014). The cellular immune response is important to monitor for a successful transplant and flow cytometry allows for measurement of this cellular response. Specifically, polyfunctional antigen-specific T cells can be protective in the patient's immune response and therefore become a barometer of transplant health. Additionally, flow cytometry may have use for analysis of CMV- and EBV-specific cells (along with cytokine formation within these cells), in order to provide a person's risk of susceptibility to major infections (CMV and EBV) that can impact whether the transplantation and graft will remain successful (Maguire et al., 2014).

Flow cytometry as a laboratory technique can measure and assess DNA ploidy through cell cycle analysis. DNA synthesis and replication errors are associated with cancer. Cancer is the uncontrolled growth and spread of abnormal cells and is increasingly shown to be initiated, propagated, and maintained by somatic genetic events (Johnson et al., 2014). Measuring ploidy is also of use when it comes to gestational trophoblastic disease, during which a group of tumors form in an abnormal pregnancy. Most gestational trophoblastic disease tumors are benign, but some have the potential to turn cancerous; usually, they are classified into two categories: hydatidiform moles and gestational trophoblastic neoplasia. Ploidy analysis through flow cytometry can help differentiate diploid from triploid conceptions, but cannot distinguish between a complete mole and a diploid nonmolar miscarriage or molar and nonmolar triploid (Horowitz et al., 2021; Seckl et al., 2013)

During the cell cycle, DNA synthesis is tightly regulated and only performed just as the cell is about to divide. This step of DNA replication is called the "S-phase" (Christensen, 2024). Dysfunction of DNA replication is significantly associated with cancer, and cancers frequently involve damage or removal of molecular regulators of replication (Van der Aa et al., 2013). Assessment of the fraction of cells in S-phase has been proposed as an indicator of neoplasm aggression. S-phase fraction (SPF) is thought to reflect proliferative activity of cancer and may provide prognostic or therapeutic information (Ermiah et al., 2012). Elevated proliferative activity may predict a worsened disease-free or overall survival in several cancers, such as breast, non-small cell lung, colorectal, ovarian, kidney, bladder, prostate, and endometrial cancers (Bagwell et al., 2001; Gawrychowski et al., 2003; Kenney et al., 2008; Mangili et al., 2008; Pinto et al., 2011; Ross, 1996). However, data supporting the use of SPF as a prognostic tool appears to be inconsistent at best (Locker et al., 2006).

Clinical Utility and Validity

Technically, any biologic sample can be analyzed by flow cytometry. However, blood is the most common sample type, including both whole blood and peripheral blood mononuclear cells (Verbsky & Routes, 2023). Flow cytometry can be employed for prognostic and diagnostic purposes. This technique has been used to identify both primary immunodeficiencies and secondary or acquired immunodeficiencies such as HIV (Verbsky & Routes, 2023). Primary immunodeficiencies represent more than 300 known genetic disorders, and flow cytometry is a major component of the diagnosis of these

disorders (Abraham & Aubert, 2016). Flow cytometry may also be used for prenatal diagnoses, hematology, transplantation, crop improvement, sperm sorting for sex preselection, post-bone marrow transplantation analyses, and during immunosuppression and chemotherapy treatments (Halder et al., 2017; Verbsky & Routes, 2023).

Today, many assays have been developed for flow cytometry purposes. These assays can identify biomarkers for cancer and stem cells, DNA and RNA, reactive oxygen species, and the functional status of yeast or bacteria (Robinson & Roederer, 2015). Newer techniques have also been developed such as mass cytometry: the combination of flow cytometry and mass spectrometry (Cosma et al., 2017). Flow fluorescent in situ hybridization (FISH) is another combinatory technique which is the combination of fluorescent in situ hybridization in suspension (FISHIS) and flow cytometry using DNA or gene-specific probes.

Flow cytometry techniques have been used to identify several types of cancer. Fromm et al. (2009) used flow cytometry to identify classical Hodgkin lymphoma, neoplastic Hodgkin, and Reed Sternberg cells in lymph nodes with 88.7% sensitivity and 100% specificity. Paiva et al. (2016) state that next generation multiparameter flow cytometry "should be considered mandatory in the routine evaluation of multiple myeloma patients both at diagnosis and after therapy and represents an attractive technique to integrate with high-throughput DNA and RNA-seq methods to help in understanding the mechanisms behind dissemination and chemoresistance of multiple myeloma." Finally, Novikov et al. (2019) used flow cytometry immunophenotyping to identify malignant T-cell clones in mature peripheral T-cell lymphomas with 97% sensitivity and 91% specificity.

Wang et al. (2019) published a study on the applicability of multiparameter (multicolor) flow cytometry (MFC) for detecting MRD to predict relapse in patients with AML after allogeneic transplantation. The researchers also compared MFC to MRD status determined using real-time quantitative polymerase chain reaction (RT-qPCR) from 158 bone marrow samples from 44 different individuals. "Strong concordance was found between MFC-based and RT-qPCR-based MRD status ($\kappa = 0.868$)." Moreover, for individuals in complete remission (CR), "the positive MRD status detected using MFC was correlated with a worse prognosis [HRs (*P* values) for relapse, event-free survival, and overall survival: 4.83 (<0.001), 2.23 (0.003), and 1.79 (0.049), respectively]; the prognosis was similar to patients with an active disease before HSCT [hematopoietic stem cell transplantation]" (Wang et al., 2019).

Jin et al. (2024) summarizes the recent progress in systemic chronic active Epstein-Barr virus (CAEBV) infection diagnosis and the utility of flow cytometry as a tool in this diagnosis. Systemic Epstein-Barr virus can have a challenging prognosis, ranging from asymptomatic to death within a few weeks. Many treatment strategies are currently ineffective and only allogenic hematopoietic stem cell transplantation is curative. The early diagnosis of systemic CAEBV could be potentially improved by examining NK/T cells using flow cytometry, effectively checking their immunological status. Flow cytometry is used to obtain as many targeted cells as possible and analyze cell size, cytoplasmic granularity, and differentiation antigens; in some cases, "the aberrant T/NK-cell population" found in CAEBV comprises less than 5% of cells in the bone marrow, and "these subtle changes may be detected via flow cytometry analyses only." However, the authors caveat, "although [flow cytometry is] good at exploring the status of NK/T cells holistically, its application to CAEBV has been limited because of the presence of overlapping antibodies and a lack of comprehensive analysis studies." As of now, flow cytometry is "mostly used to exclude lymphoma or leukemia in CAEBV patients." However, CAEBV is a progressive disease that can become extranodal NK/T lymphoma or aggressive NK-Cell leukemia and a combination of clinical features and patient outcomes may help in earlier diagnosis of ANKL or ENKTL. Flow

cytometry could, in the future, help indicate different NK Cell subtypes and differentiate the source of neoplasms (Jin et al., 2024).

Clinical Utility and Validity of DNA Ploidy Cell Cycle Analysis

Carloni et al. (2017) evaluated the associations between SPF and peritoneal carcinomatosis from ovarian cancer. Fifty-three patients were examined, and although SPF differed among the different ploidy categories, no significant correlation was found between SPF and clinical pathological characteristics of patients. However, the authors did find that sensitivity to taxol was correlated with SPF, therefore concluding that "ploidy and SPF could facilitate the choice of therapy for patients with peritoneal carcinomatosis" (Carloni et al., 2017).

Svanvik et al. (2019) examined 1113 patients diagnosed with stage I-III grade 1-3 endometrioid endometrial carcinoma in 2006-2011. They evaluated both DNA ploidy and SPF and set the SPF cutoff at eight percent. The authors found that five year relative survival was significantly associated with SPF and DNA ploidy through a univariate statistical analysis. However, when other variables such as age, grade, and stage were added, SPF and DNA ploidy became statistically insignificant. Therefore, the authors concluded that "S-phase fraction, DNA ploidy, and p53 overexpression did not improve identification of high-risk patients by stage, grade, and age in stage I-III endometrioid endometrial carcinoma" (Svanvik et al., 2019).

Thomas et al. (2020) completed a study to analyze the prognostic implications of DNA repair, DNA ploidy and telomerase in the malignant transformation risk assessment of leukoplakia. Samples from 200 patients with oral leukoplakia, 100 patients with oral cancer and 100 healthy controls were analyzed. The DNA ploidy content was measured with high resolution flow cytometry; the authors identified that "There was significant difference in the distribution of ploidy status, telomerase activity and DNA repair capacity among control, leukoplakia and oral cancer group ($p<0.001$). When the molecular markers were compared with histological grading of leukoplakia, both DNA ploidy analysis and telomerase activity showed statistical significance ($p<0.001$)" (Thomas et al., 2020).

Taniguchi et al. (2021) investigated the correlation between flow cytometry parameters such as DNA ploidy, DNA index and S-phase fraction and clinical prognostic factors such as mitotic count and Ki-67 labelling index (LI). The cancer of interest was gastrointestinal stromal tumors (GIST) and eighteen specimens from laparoscopic local gastrectomy were analyzed. The authors found these flow cytometry parameters to correlate well with mitotic count ≤ five and Ki-67 LI ≤ six. DNA index was found to be 83.3% accurate in predicting mitotic count ≤ five and 77.8% accurate in predicting Ki-67 LI ≤ six, while S-phase fraction was found to be 94.4% accurate and 88.9% accurate, respectively. The authors concluded that "Rapid flow cytometry parameters can classify risk without the need for histological analysis" (Taniguchi et al., 2021).

Panwar et al. (2021) studied the evaluation of DNA ploidy and S-phase fraction in fine needle aspirates from breast carcinoma. Fifty breast cancer patients who underwent fine needle aspiration cytology (FNAC) were included in the study. The samples from FNAC underwent DNA ploidy and SPF analysis and Ki-67 was estimated. SPF and Ki-67 were compared with each other. "On DNA flow cytometry, 27 (54%) cases were aneuploid and 23 (46%) cases were diploid. The median SPF was 12.43% and 4.03% in aneuploid and diploid tumors respectively. Median Ki-67 among aneuploid tumors was 28.6% compared to 8.7% among diploid tumors. Aneuploid tumors were significantly associated with higher values of SPF and Ki-67, with Kappa 0.437 and agreement of 72%. Diploid tumors showed lower values of SPF and Ki-67, with Kappa 0.455 and agreement of 72.7%. Correlation among SPF and Ki-67 was highly significant

with Kappa value 0.446, P value of .002 and agreement of 72.3%" (Panwar et al., 2021). The authors conclude that DNA ploidy and proliferative activity by flow cytometric SPF estimation can provide valuable prognostic information in breast cancer diagnosis.

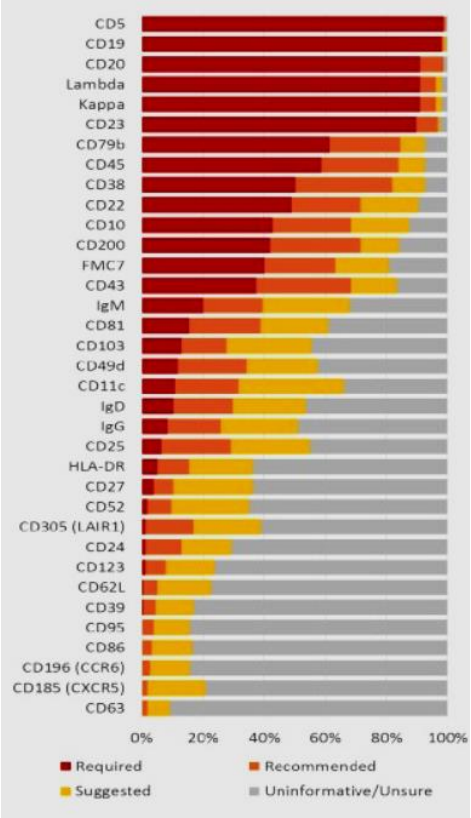
Guidelines and Recommendations

Flow cytometry is broadly used for many conditions such as cancers, which are mentioned across many different societies. The below section is not a comprehensive list of guidance for flow cytometry.

The European Research Initiative on CLL (ERIC) & European Society for Clinical Cell Analysis (ESCCA) Harmonisation Project

This group has published guidelines on chronic lymphocytic leukemia (CLL) in hopes to determine "35 potential flow cytometry markers as being "required," "recommended," "suggested," "uninformative," or "not sure" for the diagnosis of CLL (Rawstron et al., 2018). A marker is required if >75% of ERIC/ESCCA members determine that it should be required, and a marker is pushed forward for review if >50% of all members determine that it should be recommended or required. Results are shown in the following figure:

Figure 1 [taken from (Rawstron et al., 2018)]:



International/European Leukemia Net Working Group for Flow Cytometry in Myelodysplastic Syndromes

An international working party was organized to develop flow cytometry techniques in the classification of myelodysplastic syndromes (MDS). The group has stated the following guidelines:

- "In laboratories where comprehensive immunophenotyping can be performed, an MDS immunophenotyping panel… is recommended.
- In patients with cytological findings suggesting MDS of RCUD (refractory anemia subtype) or refractory anemia with ringed sideroblasts categories, aberrant flow cytometry (FCM) findings in the granulopoietic or myelomonocytic lineages may indicate multilineage dysplasia, which is of prognostic significance. Morphological findings in these cases should be thoroughly re-evaluated to avoid misclassification.
- It is important to note even small populations of myeloid progenitors with multiple immunophenotypic aberrant features (such as aberrant expression of CD7, CD56 or CD11b, see Table 1), since they indicate a higher risk of progression to AML. FCM findings in these cases should be included in the individual risk assessment" (Porwit et al., 2014).

The Clinical Cytometry Society 2006 Bethesda International Consensus

In 2006, a panel of subject matter experts convened to define the clinical indications that warrant the use of flow cytometry, as well as to identity of the reagents that should be used in the initial and secondary evaluations for those conditions (Davis et al., 2007). The output of that gathering was the 2006 Bethesda International Consensus Recommendations on the Immunophenotypic Analysis of Hematolymphoid Neoplasia by Flow Cytometry. The panel indicated that flow cytometry is useful for the evaluation of cytopenias, elevated leukocyte count, observation of atypical cells or blasts and evaluation of body fluids, plasmacytosis or monoclonal gammopathy, organomegaly and tissue masses, and certain patient monitoring indications.

The Bethesda recommendations indicate that flow cytometry is not indicated for mature neutrophilia, polyclonal hypergammaglobulinemia, polycythemia, thrombocytosis, and basophilia because "they are usually not associated with hematolymphoid malignancy or associated with hematolymphoid neoplasms that are not detectable by" flow cytometry.

The Bethesda recommendations also indicate that selection of reagents for the initial evaluation panel should be based on specimen type (peripheral blood, bone marrow, tissue, etc.), clinical information and cell morphology studies. They identify initial panels for specific indications that range from a total of four reagents to a maximum of 12 reagents.

For secondary evaluation, where the initial evaluation is not conclusive or informative, the Bethesda recommendations again identify groups of reagents that should be used, based on indication. The secondary panels ranged from five to 23 reagents.

Specific recommendations for the initial evaluation were:

- B cells: CD5, CD10, CD19, CD20, CD45, Kappa, Lambda
- T cells and NK cells: CD2, CD3, CD4, CD5, CD7, CD8, CD45, CD56
- Myelomonocytic cells: CD7, CD11b, CD13, CD14, CD15, CD16, CD33, CD34, CD45, CD56, CD117, HLA-DR
- Myelomonocytic cells (limited): CD13, CD33, CD34, CD45
- Plasma cells CD19, CD38, CD45, CD56

For secondary evaluation, the Bethesda recommendations were:

- B cells: CD9, CD11c, CD15, CD22, cCD22, CD23, CD25, CD13, CD33, CD34, CD38, CD43, CD58, cCD79a, CD79b, CD103, FMC7, Bcl-2, cKappa, cLambda, TdT, Zap-70, cIgM
- T cells and natural killer (NK) cells: CD1a, cCD3, CD10, CD16, CD25, CD26, CD30, CD34, CD45RA, CD45RO, CD57, ab-TCR, gd-TCR, cTIA-1, T-beta chain isoforms, TdT
- Myelomonocytic cells: CD2, CD4, CD25, CD36, CD38, CD41, CD61, cCD61, CD64, CD71, cMPO, CD123, CD163, CD235a
- Plasma cells: CD10, CD117, CD138, cKappa, cLambda

The American Society of Clinical Oncology Tumor Markers Expert Panel (ASCO)

In 2006, the ASCO updated the recommendations for the use of tumor marker tests in the prevention, screening, treatment, and surveillance of gastrointestinal cancers. These recommendations state that "Neither flow-cytometrically derived DNA ploidy (DNA index) nor DNA flow cytometric proliferation analysis (% S phase) should be used to determine prognosis of early-stage colorectal cancer" (Locker et al., 2006). This guideline also stated that for now, flow cytometric determination of DNA ploidy or proliferation should, at best, be considered an experimental tool.

In 2007, the ASCO updated the recommendations for the use of tumor marker tests in the prevention, screening, treatment, and surveillance of breast cancer (Harris et al., 2007); the authors noted that "DNA/ploidy by flow cytometry demonstrated insufficient evidence to support routine use in clinical practice."

College of American Pathologists and the American Society of Hematology

In 2016, the College of American Pathologists (CAP) and the American Society of Hematology (ASH) published a joint guideline to outline their recommendations for the initial diagnostic workup of acute leukemia. Among their 27 recommendations, three statements (each rated "Strong Recommendation") explicitly address the leveraging of flow cytometry in said process:

"5. In addition to morphologic assessment (blood and bone marrow), the pathologist or treating clinician should obtain sufficient samples and perform conventional cytogenetic analysis (i.e., karyotype), appropriate molecular genetic and/or fluorescent in situ hybridization (FISH) testing, and flow cytometric immunophenotyping (FCI). The flow cytometry panel should be sufficient to distinguish acute myeloid leukemia (including acute promyelocytic leukemia), T-cell acute lymphoblastic leukemia (T-ALL) (including early T-cell precursor leukemias), B-cell precursor ALL (B-ALL), and acute leukemia of ambiguous lineage on all patients diagnosed with acute leukemia. FISH and/or molecular genetic testing does not, however, replace conventional cytogenetic analysis.

Note — If sufficient bone marrow aspirate or peripheral blood material is not available for FCI, immunohistochemical studies may be used as an alternative method for performing limited immunophenotyping. In addition, a second bone marrow core biopsy can be obtained and submitted, unfixed in tissue culture media, for disaggregation for genetic studies and flow cytometry."

"10. For patients with suspected or confirmed acute leukemia, the pathologist may use flow cytometry for the evaluation of CSF."

"12. For patients with suspected or confirmed acute leukemia, the pathologist or treating clinician should ensure that flow cytometry analysis or molecular characterization is comprehensive enough to allow subsequent detection of minimal residual disease."

A final recommendation (also a "Strong Recommendation") mentioning flow cytometry referred to the use of its data, such that

"24. If a patient is referred to another institution for treatment, the primary institution should provide the treatment center with all laboratory results, pathology slides, flow cytometry data, cytogenetic information, and a list of pending tests at the time of the referral. Pending test results should be forwarded when they become available" (D. Arber, 2017).

International Society on Thrombosis and Haemostasis (ISTH)

The International Society on Thrombosis and Haemostasis SSC Subcommittee outlined several recommendations for which flow cytometric analysis of inherited and acquired platelet disorders should occur. Those clinical settings in which it believed would be helpful, guided by expert consensus, are reported below:

- "Diagnosis of inherited or acquired deficiencies of platelet surface glycoproteins (BSS, GT, inherited or immune-mediated GPVI defects)
- Diagnosis of platelet alpha granule secretion defects (such as gray platelet syndrome)
- Diagnosis of defects in specific platelet activation (signaling) pathways (such as RASGRP2, P2Y12, or TXA2R disorders)
- Diagnosis of GFI1B macrothrombocytopenia associated to platelet expression of CD34
- Diagnosis of disorders of platelet procoagulant activity (such as Scott syndrome and Stormorken syndrome)
- Assessment of increased platelet activation in prothrombotic syndromes (diabetes, anti-phospholipid syndrome or secondary to drug induced, non-immune platelet activation)
- Monitoring, if applicable, pharmacodynamic effect of P2Y12 antagonists (ticlopidine, clopidogrel, prasugrel, ticagrelor, cangrelor) with specifically designed test such as VASP P2Y12
- Determination of the fraction of immature platelets" (Frelinger et al., 2021).

National Comprehensive Cancer Network (NCCN)

NCCN clinical practice guidelines on diagnosis and/or management of Breast Cancer (Version 4.2024), Cervical Cancer (Version 3.2024), Colon Cancer (Version 4.2024), Small Cell Lung Cancer (Version 3.2024), and Non-Small Cell Lung Cancer (Version 7.2024) do not mention cell proliferation activity (S-phase fraction or % S-phase) as a management tool (NCCN, 2024).

International Society of Gynecological Pathologists (ISGyP) Endometrial Cancer Project: Guidelines from the Special Techniques and Ancillary Studies Group

These guidelines focus on biomarkers and their potential use for endometrial carcinoma.

The guideline remarks that "Other than markers which are useful in diagnosis, there are few specific studies that provide definitive evidence for the routine use of IHC [immunohistochemistry] or ploidy analysis in determining the prognosis of EC" and that "There is some literature on the association of ploidy with prognosis, with promising results, but there is a lack of definitive studies to determine its true prognostic impact."

Overall, the guideline states that "Clearly, large prospective, well defined, uniform studies are needed to determine the possible role of IHC for specific biomarkers and ploidy analysis in the clinical setting" (Cho et al., 2019).

Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
86355	B cells, total count
86356	Mononuclear cell antigen, quantitative (eg, flow cytometry), not otherwise specified, each antigen
86357	Natural killer (NK) cells, total count
86359	T cells; total count
86360	T cells; absolute CD4 and CD8 count, including ratio
86361	T cells; absolute CD4 count
86367	Stem cells (ie, CD34), total count
88182	Flow cytometry, cell cycle or DNA analysis
88184	Flow cytometry, cell surface, cytoplasmic, or nuclear marker, technical component only; first marker
88185	Flow cytometry, cell surface, cytoplasmic, or nuclear marker, technical component only; each additional marker (List separately in addition to code for first marker)
88187	Flow cytometry, interpretation; 2 to 8 markers
88188	Flow cytometry, interpretation; 9 to 15 markers
88189	Flow cytometry, interpretation; 16 or more markers

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

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Revision History

Revision Date	Summary of Changes
09/04/2024	Reviewed and Updated: Updated background, guidelines, and evidence-based scientific references. Literature review necessitated the following changes in coverage criteria: Addition of "preoperative" to CC1f. Now reads: "f) For preoperative or post-operative monitoring of individuals who will undergo or who have undergone organ transplantation." Removed CC3 pertaining to code information, as this does not fit the traditional format of Avalon coverage criteria and does not affect enforcement within this policy.

Folate Testing

Policy Number: AHS – G2154 – Folate Testing	Prior Policy Name and Number, as applicable:
Initial Presentation Date: 06/11/2018 Revision Date: February 1, 2025	

Policy Description

Folate, or vitamin B9, is a generic term for a water-soluble vitamin obtained from the diet that is involved in the transfer of methyl groups (i.e., single carbon-containing groups) in multiple biochemical metabolic pathways, including nucleic acid biosynthesis and methionine/homocysteine metabolism. Folate metabolism is closely linked to vitamin B12, cobalamin. Folate deficiency can be implicated in many disease states and processes; however, it is usually easily remedied with either a change in diet or a dietary supplement of the synthetic form, folic acid (Means Jr & Fairfield, 2023a; NIH, 2018).

Related Policies

Policy Number	Policy Title
N/A	

Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) For individuals diagnosed with megaloblastic or macrocytic anemia **and** for whom the megaloblastic anemia and/or macrocytosis does not resolve after folic acid treatment, measurement of serum folate concentration **MEETS COVERAGE CRITERIA**.

2) For all indications not described above, measurement of serum folate concentration **DOES NOT MEET COVERAGE CRITERIA**.

3) For all indications, measurement of red blood cell (RBC) folate **DOES NOT MEET COVERAGE CRITERIA**.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

4) For all situations, folate receptor autoantibody testing **DOES NOT MEET COVERAGE CRITERIA.**

Table of Terminology

Term	Definition
AA	Aplastic anemia
AACE	American Association of Clinical Endocrinologists
AAFP	American Academy of Family Physicians
AAN	American Academy of Neurology
ABIM	American Board of Internal Medicine
ACE	American College of Endocrinology
ACG	American College of Gastroenterology
AND	Academy of Nutrition and Dietetics
ApABG	*Para*-acetamidobenzoylglutamate
ASA	American Society of Anesthesiologists
ASCP	American Society for Clinical Pathology
ASMBS	American Society for Metabolic and Bariatric Surgery
ASPEN	American Society for Parenteral and Enteral Nutrition
BCMA	British Columbia Medical Association
BCSH	British Committee for Standards in Haematology
CBC	Complete blood count
CD	Celiac disease
CDC	Centers for Disease Control and Prevention
CKD	Chronic kidney disease
CLIA '88	Clinical Laboratory Improvement Amendments of 1988
CMS	Centers for Medicare and Medicaid
CPT	Current Procedural Terminology
CRP	C-reactive protein
DHFR	Dihydrofolate reductase
DNA	Deoxyribonucleic acid
ECCO	European Crohn's and Colitis Organization
EL	Evidence level
FDA	Food and Drug Administration
Hb	Hemoglobin
HCPCS	Healthcare Common Procedure Coding System
IBD	Irritable bowel disorders
IOM	Institute of Medicine
KDIGO	Kidney Disease Improving Global Outcomes
LDT	Laboratory-developed test

Term	Definition
MCG	Microgram
MCMs	Major congenital malformation
MMWR	Morbidity and mortality weekly report
MTHFR	Methylenetetrahydrofolate reductase
NBA	Australian National Blood Authority
NCCN	National Comprehensive Cancer Network
NHANES	National Health and Nutrition Examination Survey
NICE	National Institute for Health and Care Excellence
NTDs	Neural tube defects
OMA	Obesity Medicine Association
PA	Prior authorization
pABG	*Para*-aminobenzoylglutamate
RBC	Red blood cell
RDN	Registered dietitian nutritionist
RF	Red blood cell folate testing
SCCM	Society of Critical Care Medicine
SF	Serum folate testing
SSCM	Society of Critical Care Medicine
TOS	The Obesity Society
TSAT	Transferrin saturation
UL	Upper limits
USPSTF	United States Preventive Services Task Force

Scientific Background

Folate, or vitamin B9, naturally occurs as polyglutamated compounds (pteroylpolyglutamates) in many plant and animal products. The synthetic form is a monoglutamate-containing compound called folic acid. Folic acid is more chemically stable for commercial production and storage, but it is less bioavailable than the naturally occurring folate (Means Jr & Fairfield, 2023a). Biochemically, folate is a coenzyme in single-carbon transfers *in vivo* and is directly linked to the cobalamin (vitamin B12) cycle, methionine metabolism, and nucleic acid biosynthesis. Dietary folates are hydrolyzed via γ-glutamyl hydrolase (or folate conjugase) prior to absorption in the intestinal mucosa (IOM, 1998). Both folate and vitamin B12 are required for formation of 5,10-methylene tetrahydrofolate, which is the cofactor involved in purine synthesis. Methylenetetrahydrofolate reductase (MTHFR) is the enzyme responsible in converting 5,10-methylene tetrahydrofolate to 5-methyl tetrahydrofolate, which is required for methionine synthase, the enzyme that converts homocysteine to methionine. The interlinked one-carbon cycle is depicted in the figure below with the metabolites assayed in clinical laboratories in bold (Finer et al., 2013).

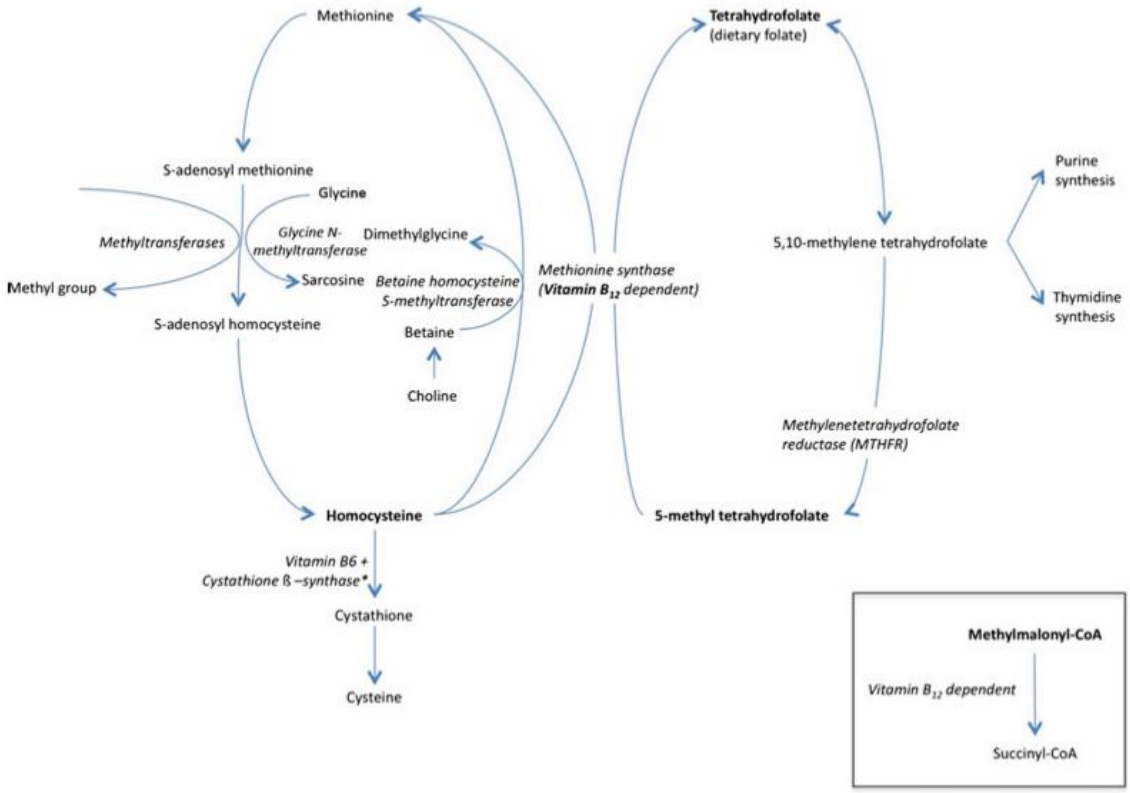

FIGURE 1 The one-carbon cycle. Metabolites readily assayed in clinical laboratories are highlighted in bold.

Role of Folate in Anemia

Anemia occurs when the body lacks healthy red blood cells (RBCs), leading to an insufficient amount of oxygen delivered to tissues. Typical symptoms of anemia include fatigue, weakness, pale skin, and lightheadedness.

Macrocytic anemia refers to anemias that have high mean corpuscular volume with large RBCs. Mean corpuscular volume, or mean cell volume, can be defined as the average volume of RBCs in an individual. Megaloblastic anemia is a specific macrocytic anemia due to nucleic acid metabolic defects

that result in "nuclear-cytoplasmic dyssynchrony, reduced number of cell divisions in the bone marrow, and nuclear abnormalities in both myeloid and erythroid precursors" caused by folate and/or vitamin B12 deficiency (Means Jr & Fairfield, 2023b). These abnormal RBCs are the principle clinical manifestations of folate deficiency and symptoms "include weakness, fatigue, difficulty concentrating, irritability, headache, heart palpitations, and shortness of breath" (NIH, 2018).

Folate and Neural Tube Defects (NTDs)

Neural tube defects (NTDs) develop early in pregnancy and are malformations of the brain and/or spine that include spina bifida and anencephaly. Folate deficiency is directly linked to NTDs. The role of folate in NTD development is not well-characterized. The role of folate in either the methylation cycle or nucleic acid synthesis has been suggested to play a part in NTD development during embryogenesis, and some studies have indicated that it is the bioavailability of specific folates in the pregnant individual that can increase the likelihood of NTDs (Imbard et al., 2013; Rothenberg et al., 2004). Individuals typically do not obtain enough folate from diet alone, so individuals of childbearing age are recommended to take a synthetic folic acid supplement to decrease the likelihood of NTDs in offspring (Bibbins-Domingo et al., 2017). To decrease the occurrence of NTDs and folate deficiency, the United States and Canada mandated folic acid supplementation to cereal grains in 1998, and as of March 2018 "92 countries have legislation to mandate fortification of at least one industrially milled cereal grain" (FFI, 2021).

It is notable that the prevalence of folate deficiency, and the prevalence of NTDs has declined in countries with routine folic acid supplementation (Crider et al., 2011). A review by Imbard et al. (2013) of 17 different studies on the impact of folic acid fortification of NTD rates show that 16 show a decrease in the rate of NTDs. Only one study of the rate of NTDs in California showed no decline since fortification. The reduction of the United States overall was 26-30% since folic acid fortification (Imbard et al., 2013).

Folate Receptor Antibody Testing (FRAT®)

Folate deficiency in the pregnant individual can "lead to pregnancy-related complications including neural tube defects (NTDs) in the fetus. Numerous studies have now established the benefits of folate supplementation in reducing the incidence of NTD pregnancy" (Sequeira, 2012). Fratnow's FRAT® measures the "presence of antibodies that interact by either blocking or binding with the activity of the Folate Receptor A. Data shows that folate is critical for the proper function of many tissues, including brain, placenta, and ovaries. FRAT® is not indicated for the diagnosis of any medical condition and thus has not been approved by the FDA. FRAT® can be useful as a research tool in the above disorders, as well as assessing the health of folate transport to the brain, placenta, and ovary" (Fratnow, 2016).

Causes of Folate Deficiency

Folate deficiency can be caused by dietary intake. Nutritional deficits may occur due to diet, alcoholism, depression, and even overcooked foods. Many malabsorptive disorders, such as celiac disease and ulcerative colitis, can also result in a decrease in folate uptake. Further, bariatric procedures may result in decreased absorption, and drugs, including methotrexate and trimethoprim that inhibit dihydrofolate reductase (DHFR), can also cause a folate deficiency. It is also important to note that an increased need of folate for DNA synthesis during pregnancy and lactation, chronic hemolytic anemias, exfoliative skin diseases, and hemodialysis cause folic acid deficiency. Folate deficiency is also more prevalent in older adults than younger (Means Jr & Fairfield, 2023b).

Methodology of Folate Testing

Folate concentrations have been measured from serum, erythrocytes (RBC), and urine. Serum folate levels may not "differentiate between what may be a transitory reduction in folate intake or chronic folate deficiency accompanied by depleted folate stores and functional changes" (IOM, 1998). RBCs have a lifespan of approximately 120 days, and folate is only taken in during initial erythropoiesis (red blood cell production); consequently, RBC folate concentrations are less likely to be affected by transitory dietary fluctuations. However, Wu et al. (1975) show that both RBC folate and serum folate levels correlate to hepatocyte folate levels (IOM, 1998; Wu et al., 1975). Galloway and Rushworth (2003) released a study in conjunction with the National Pathology Alliance review in the United Kingdom comparing data of laboratories of the National Health Service that routinely use serum folate testing only, RBC folate testing only, or both serum and RBC folate testing together. The researchers conclude that there is no need to use both tests to determine folate concentration as an initial screen. "The serum folate assay provided equivalent information to the measurement of red cell folate and evidence from the literatures [sic] suggest that the serum folate assay should be the method of choice" (Galloway & Rushworth, 2003).

Clinical Utility and Validity

A study by Shojania and von Kuster (2010) investigated the use of serum folate testing (Trompeter et al.) and RBC folate testing (RF) in cases of anemia in a country that has mandated folic acid supplementation in grain products. By examining the data for folate testing in anemia at two different teaching hospitals in Canada, they report that in one hospital in 2001 "11 out of 2154 (0.5%) SF were low (<7.0 nmol/L) and 4 out of 560 (0.7%) RF were low (<417 nmol/L). In no subject with low SF or RF could the anemia be attributed to folate deficiency." For the other hospital, the data from 1999-2001 shows that "19 out of 991 (1.9%) had low RF (<225 nmol/L) but in only 2 patients (0.2%) the low RF was in folate deficiency anemia range" (Shojania & von Kuster, 2010). The authors conclude that neither serum folate testing, nor RBC folate testing is justified in cases of anemia for folic acid fortified countries due to such low incidence rates of folate deficiency anemia.

A study by Joelson et al. (2007) examined the records of three different hospitals in the U.S. that service a high number of indigent patients. The researchers reported the data from three non-consecutive years (1997, 2000, and 2004) to examine the impact of folate fortification in food products. Using the RBC folate levels only with a RBC folate cutoff value of 160 ng/mL (363.6 nmol/L), "the combined incidence of folate deficiency decreased from 4.8% in 1997 to 0.6% in 2004…Even when the folate concentration was found to be low, the majority of these subjects did not have macrocytosis." This study included a total of 4134 RBC folate tests performed over the course of three years. It is of interest to note that the number of tests performed increased from 813 in 1997 to 1759 in 2004. The authors do note of a potential limitation of the study since the data of the patients cannot be separated into specific groups (pregnant individuals, alcoholics, socioeconomic classes, and so on). The authors conclude "that folate deficiency has become a rare event in the United States, and the utility of routine folate measurements for patients with anemia and/or increased mean corpuscular volume are difficult to justify" (Joelson et al., 2007).

Urinary folate levels do not reflect either the stored folate concentrations or the fluctuations in folate concentration due to transitory dietary changes. Only about 1-2% of the folate excreted in the urine is unmetabolized and "excretion continued in the face of advanced folate depletion" (IOM, 1998). One study of ten postmenopausal individuals on a low folate diet measured folate turnover using urinary testing of folate and folate metabolites. "Folate intake did not significantly influence ApABG (*para*-acetamidobenzoylglutamate) or pABG (*para*-aminobenzoylglutamate) excretion." ApABG and pABG along with pterins are the major folate catabolites. The authors conclude that "the rate of folate

catabolite excretion is related mainly to masses of slow-turnover folate pools governed by long-term folate intake" (Gregory et al., 2000).

Epstein-Peterson et al. (2020) collected and analyzed all folate tests performed in 2017 at an academic cancer center. In total, 937 patients were tested 1065 times; approximately 7% of tests indicated a folate deficiency, and folate deficiency was significantly associated with a higher risk of death (P=0.01) (Epstein-Peterson et al., 2020).

Tran et al. (2022) performed a literature review on the diagnostic accuracy, clinical utility, cost-effectiveness, and evidence-based guidelines regarding the use of serum folate testing in people with suspected folate deficiency. An information specialist completed a literature search using the search concepts "folate deficiency AND testing" and only limiting results to the human population for publications between January 1, 2012, and February 15, 2022. The authors were not able to identify any relevant literature regarding diagnostic test accuracy, clinical utility, cost-effectiveness, or evidence-based guidelines (Tran et al., 2022).

Guidelines and Recommendations

Centers for Disease Control and Prevention (CDC)

The CDC urges all individuals who are capable of becoming pregnant and who are also of reproductive age to "take 400 micrograms (Handelsman et al.) of folic acid each day, in addition to consuming food with folate from a varied diet, to help prevent some major birth defects of the baby's brain (anencephaly) and spine (spina bifida)" (CDC, 2022). This was reviewed in 2021. This recommendation includes all individuals of reproductive age planning to become pregnant or not, as about half of U.S. pregnancies are unplanned.

American Society for Clinical Pathology (ASCP)/Choosing Wisely

The ASCP published a recommendation in 2017 in Choosing Wisely, an American Board of Internal Medicine (ABIM) initiative, where they clearly state the following: "Do not order red blood cell folate levels at all. In adults, consider folate supplementation instead of serum folate testing in patients with macrocytic anemia." They indicate that the drastic decrease in folic deficiency in both the U.S. and Canada after mandated folic acid supplementation in foods no longer requires for either serum folate or red blood cell folate concentrations be tested. "While red blood cell folate levels have been used in the past as a surrogate for tissue folate levels or a marker for folate status over the lifetime of red blood cells, the result of this testing does not, in general, add to the clinical diagnosis or therapeutic plan" (ASCP, 2017).

National Pathology Alliance (of the United Kingdom)

The National Pathology Alliance of the United Kingdom in 2003 published in the *Journal of Clinical Pathology* their recommendation "that serum folate measurements provide equivalent information to red cell folate measurements" (Galloway & Rushworth, 2003).

American Association of Clinical Endocrinologists (AACE)/The American College of Endocrinology (ACE), The Obesity Society (TOS), American Society for Metabolic and Bariatric Surgery (ASMBS), Obesity Medicine Association (OMA), and American Society of Anesthesiologists (ASA)

In 2013, the AACE, ACE, and TOS issued joint guidelines regarding healthy eating for the prevention and treatment of metabolic and endocrine diseases in adults (Gonzalez-Campoy et al., 2013). Based on the

data from the National Health and Nutrition Examination Survey (NHANES), they state "that patients with vitamin B_{12} deficiency had higher folate levels, were more likely to be anemic, and had more cognitive impairment that those with normal serum folate levels" [evidence level (EL) 2]. They evaluate the evidence concerning the link between folate and cardiovascular disease as EL4 and the link between NTDs and folate as EL1. With respect to pregnancy nutritional needs, they "should be assessed prior to conception to improve pregnancy outcome." All individuals of childbearing age "should consume at least 400 µg dietary equivalents of folate per day" [EL4] and that during pregnancy the daily amount should be increased to 600 µg [EL3].

The AACE and ACE in 2015 released their *Clinical Practice Guidelines for Developing a Diabetes Mellitus Comprehensive Care Plan* (Handelsman et al., 2015). Concerning patients with diabetic nephropathy, the suggest that they "undergo annual or more frequent assessment of electrolytes." For those with anemia, iron, transferrin saturation (TSAT), ferritin, vitamin B_{12}, and folate levels "should be further investigated" [EL4].

In 2017, the AACE and ACE released their guidelines for management of dyslipidemia and prevention of cardiovascular disease (Jellinger et al., 2017). Since bile acid sequestrant treatments such as cholestyramine can cause folate depletion in children, they recommend that children on such treatments supplement their diet with a multivitamin. They also note that folate, B6, and B12 supplementation can help mediate hyperhomocysteinemia, but that the supplements do not reduce risk of atherosclerotic cardiovascular disease.

In 2019, the AACE/ACE, TOS, ASMBS, OMA, and ASA issued joint guidelines for the perioperative nutritional, metabolic, and nonsurgical support of the bariatric surgery patient (Mechanick et al., 2019). Here, as part of a pre-operative bariatric surgery checklist that has a "Grade A" recommendation, they include "nutrient screening with iron studies, B_{12} and folic acid (RBC folate, homocysteine, methylmalonic acid optional) …consider more extensive testing in patients undergoing malabsorptive procedures based on symptoms and risks." With regards to patients who become pregnant after having a bariatric procedure, they recommend (with Grade D) having nutritional surveillance laboratory screenings done each trimester for folate deficiency along with iron, calcium, B_{12}, and vitamin D, and if after a malabsorptive procedure, fat-soluble vitamins, zinc, and copper. With a Grade C, they state that "nutritional anemias resulting from malabsorptive bariatric surgical procedures can involve deficiencies in vitamin B12, folate, protein, copper, selenium, and zinc and may be evaluated when routine aggressive case finding for iron-deficiency anemia is negative." Additionally, findings of folate deficiency in patients with obesity prior to bariatric surgery by the ASMBS "justifies aggressive case finding preoperatively with biochemical testing, specifically using sensitive markers, such as red-blood-cell folate and homocysteine (methylmalonic acid is normal with folate deficiency and normal B12 status)" and they note that particular attention should be given to individuals of childbearing age.

National Institute for Health and Care Excellence (NICE)

The National Institute for Health and Care Excellence (NICE) of the Department of Health in the United Kingdom published their extensive guidelines concerning bladder cancer on February 25, 2015. Within the section concerning the follow-up treatment for muscle-invasive bladder cancer, they recommend a protocol after radical cystectomy that includes "monitoring for metabolic acidosis and B12 and folate deficiency at least annually" (NICE, 2015). This guideline was reaffirmed in 2019.

American Academy of Family Physicians (AAFP)

The AAFP released the recommendations concerning macrocytosis and macrocytic anemia in 2009. Of note, they state that "serum folate levels are not useful because they fluctuate rapidly with dietary intake and are not cost effective. RBC folate levels more accurately correlate with folate stores and should be performed if folate deficiency is suspected." They give the following key recommendation (with evidence rating of "C" or "consensus, disease-oriented evidence, usual practice, expert opinion, or case series") to "obtain red blood cell folate level if other etiologies are not found (serum folate levels may be misleading)." In the evaluation of macrocytic anemia, they included a flowchart outlining the order of steps and tests to be taken, including when the RBC folate level should be checked. For a patient exhibiting a mean corpuscular volume 100 fL and an abnormal peripheral smear showing megaloblastic features and a reticulocyte count under 2%, they should have their RBC folate level measured only if the vitamin B_{12} level is >400 pg. The flowchart is included below (Kaferle & Strzoda, 2009).

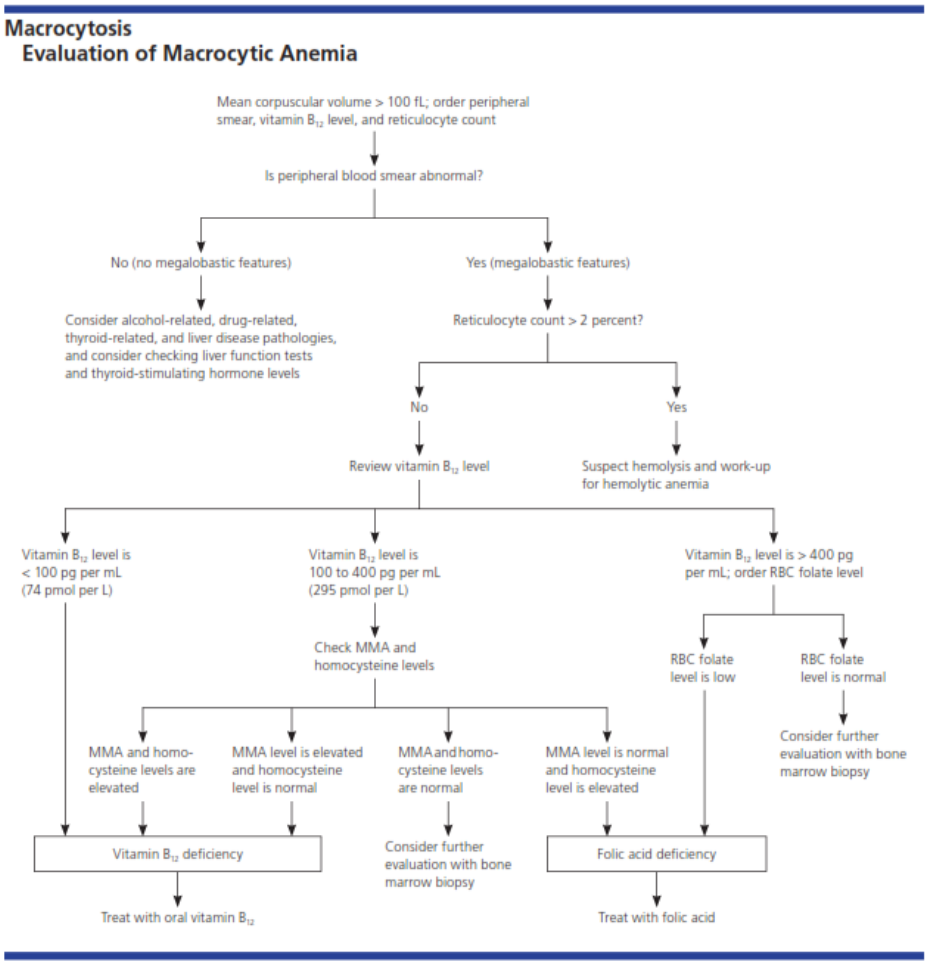

Figure 3. Algorithm for the evaluation of macrocytic anemia. (RBC = red blood cell; MMA = methylmalonic acid.)

American Academy of Neurology (AAN)

In 2001, the AAN updated their practice parameters for the diagnosis of dementia. Within the section concerning the comorbidities that should be screened in an initial assessment for dementia, they recommend folate testing along with complete blood count, serum electrolytes, B_{12}, blood urea nitrogen/creatinine, syphilis serology, thyroid function, and glucose. They did note that as of that time "no studies were identified that evaluated these recommendations" since the last practice parameters released in 1994 (Knopman et al., 2001).

Kidney Disease Improving Global Outcomes (KDIGO)

KDIGO released their updated *KDIGO Clinical Practice Guideline for Anemia in Chronic Kidney Disease* in 2012. They gave a "not graded" recommendation for "in patients with CKD [chronic kidney disease] and anemia (regardless of age and CKD stage), include the following tests in initial evaluation of the anemia (*Not Graded*):

- Complete blood count (CBC), which should include Hb concentration, red cell indices, white blood cell count and differential, and platelet count
- Absolute reticulocyte count
- Serum ferritin level
- Serum transferring saturation (TSAT)
- Serum vitamin B_{12} and folate levels"

They also state that "RBC folate levels can be measured when serum folate levels are equivocal or when there is concern that recent dietary intake may obscure underlying folate deficiency using serum levels alone" (McMurray et al., 2012).

American Society for Parenteral and Enteral Nutrition (ASPEN) & Society of Critical Care Medicine (SSCM)

In 2013, ASPEN and SSCM issued joint clinical guidelines concerning the nutrition support of hospitalized obese adults. With a "Recommendation: Weak" status, they recommended "in acutely ill hospitalized patients with history of these procedures [sleeve gastrectomy, gastric bypass, or biliopancreatic diversion ± duodenal switch], evaluation for evidence of depletion of iron, copper, zinc, selenium, thiamine, folate, and vitamins B_{12} and D is suggested as well as repletion of deficiency states" (Choban et al., 2013).

In 2016, ASPEN and SSCM issued their *Guidelines for the provision and assessment of nutrition support therapy in the adult critically ill patient*. The committee recommended that "evaluation for and treatment of micronutrient deficiencies such as calcium, thiamin, vitamin B12, fat-soluble vitamins (A, D, E, K), and folate, along with the trace minerals iron, selenium, zinc, and copper, should be considered" (McClave et al., 2016). In 2021, the ASPEN and SSCM updated their *Guidelines for the provision and assessment of nutrition support therapy in the adult critically ill patient*; the guidelines do not mention folate testing (Compher et al., 2022). In 2017, ASPEN and SSCM updated their *Guidelines for the provision and assessment of nutrition support therapy in the pediatric critically ill patient*. These guidelines do not mention folate testing (Mehta et al., 2017).

Academy of Nutrition and Dietetics (AND)

The AND released their *Oncology evidence-based nutrition practice guideline* in 2013 and reaffirmed the guideline in a 2017 publication. On the "Assessment of Biochemical Data Medical Tests, and Procedures on Adult Oncology Patients" portion, the committee recommended with "Consensus, Imperative" that "the RDN [Registered Dietitian Nutritionist] should evaluate available data and recommend as indicated: biochemical data, medical tests and procedures of adult oncology patients" and included on their list is "Nutritional anemia profile (hemoglobin, hematocrit, folate, B12, iron)." "Assessment of these factors is needed to effectively determine nutrition diagnoses and plan the nutrition interventions" (Thompson et al., 2017).

European Crohn's and Colitis Organisation (ECCO)

ECCO's guidelines concerning irritable bowel disorders (IBD) included an extensive discussion on causes and treatments of anemia in IBD—both iron deficiency anemia and non-iron deficiency anemia. With an

[EL 5], they state that "deficiencies of Vitamin B_{12} and folate should be treated to avoid anaemia. Serum levels of vitamin B_{12} and folic acid should be measured at least annually, or if macrocytosis is present. Patients at risk for vitamin B_{12} or folic acid deficiency [eg small bowel disease or resection] need closer surveillance. The recommended timelines are based on expert opinions and reflect common clinical practice, but do not apply to patients with extensive small bowel resection, extensive ileal Crohn's disease, or ileal-anal pouch" (Dignass et al., 2015).

American College of Gastroenterology (ACG)

In their guidelines and recommendations concerning the diagnosis and management of celiac disease (CDC) in 2013, the ACG recommended the following statement with *Conditional recommendation, low level of evidence*: "People with newly diagnosed CD should undergo testing and treatment for micronutrient deficiencies. Deficiencies to be considered for testing should include, but not be limited to, iron, folic acid, vitamin D, and vitamin B12" (Rubio-Tapia et al., 2013). This guideline was reaffirmed in 2016.

British Committee for Standards in Haematology (BCSH)

In 2014, the BCSH released guidelines on folate deficiencies. They noted that "routine red cell folate testing is not necessary because serum folate alone is sufficient in most cases." However, they also acknowledged that "in the presence of strong clinical suspicion of folate deficiency, despite a normal serum level, a red cell folate assay may be undertaken, having ruled out cobalamin deficiency." The BCSH also noted that "folate status is generally checked in clinical situations similar to those of cobalamin deficiency (Grade 1A)" (Devalia et al., 2014).

In 2016, the BCSH recommended that a "documented vitamin B12 or folate deficiency should be corrected before a final diagnosis of AA is confirmed. Bone marrow aplasia due to vitamin deficiency is exceedingly rare" (Killick et al., 2016).

In the 2021 BCSH *Guidelines for the Investigation and Management of Vitamin B12 and Folate Deficiency* list the following four indications for folate testing: "unexplained anaemia/macrocytic anaemia/megaloblastic anaemia, excess alcohol intake especially with coexisting liver disease, exfoliative skin diseases, post gastric and bariatric surgery." Alternatively, the guidelines list the following two indications when folate supplementation should occur without folate testing: "pregnancy, haemolytic anaemia – autoimmune haemolysis, red cell membrane disorders and haemoglobinopathies." The guidelines also state that folate and B12 should always be tested together, but notes that "once a patient has commenced B12 replacement there is no further need for it to be measured again" (BCSH, 2021).

Renal Association Clinical Practice Guideline

The Renal Association recommends measuring serum folate concentration for evaluation of anemia in CKD (Mikhail et al., 2017).

National Comprehensive Cancer Network (NCCN)

The NCCN recommends measurement of RBC folate as part of the initial evaluation for myelodysplastic syndromes. Serum folate may be considered as an alternative, but is not preferable to RBC folate. "RBC folate is a more representative measure of folate stores and is the preferred test to serum folate" (NCCN, 2023).

Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
82746	Folic acid; serum
82747	Folic acid; RBC
0399U	Neurology (cerebral folate deficiency), serum, detection of anti-human folate receptor IgG-binding antibody and blocking autoantibodies by enzyme-linked immunoassay (ELISA), qualitative, and blocking autoantibodies, using a functional blocking assay for IgG or IgM, quantitative, reported as positive or not detected Proprietary test: FRAT® (Folate Receptor Antibody Test) Lab/Manufacturer: Religen Inc

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Revision History

Revision Date	Summary of Changes
05/31/2023	Reviewed and Updated: Updated background, guidelines, and evidence-based scientific references. Literature review necessitated the following changes in coverage criteria: CC1 reorganized from having subcriteria into being a single, main CC. Now reads: "1) For individuals diagnosed with megaloblastic or macrocytic anemia and for whom the megaloblastic anemia and/or macrocytosis does not resolve after folic acid treatment, measurement of serum folate concentration MEETS COVERAGE CRITERIA." All CC edited for clarity and consistency. Addition of new CC4: "4) For all situations, folate receptor autoantibody testing DOES NOT MEET COVERAGE CRITERIA."

Revision Date	Summary of Changes
	Added PLA code 0399U.

Gamma-glutamyl Transferase Testing in Adults

Policy Number: AHS – G2173 – Gamma-glutamyl Transferase Testing in Adults	Prior Policy Name and Number, as applicable: • AHS – G2173 – Gamma-glutamyl Transferase
Initial Presentation Date: 05/26/2020 Effective Date: February 1, 2025	

Policy Description

Gamma-glutamyl transferase (GGT), also known as gamma-glutamyl transpeptidase (GGTP) (Singh et al., 2006; Vroon & Israili, 1990), is an enzyme that has a half-life of between fourteen and twenty-six days and is present in the cell membrane of many different tissue types, including the heart, brain, seminal vesicles, kidneys, bile duct, spleen, and gallbladder (Dillon & Miller, 2016; Dixit & Singh, 2015). GGT is traditionally considered a predictive marker for liver dysfunction, bile duct ailments, and alcohol consumption (Koenig & Seneff, 2015). However, new research suggests that GGT may be useful as an early predictive marker for several other conditions including heart failure, arterial stiffness, arterial plaque, gestational diabetes, atherosclerosis, several infectious diseases, and numerous types of cancer (Koenig & Seneff, 2015). Terms such as male and female are used when necessary to refer to sex assigned at birth.

Related Policies

Policy Number	Policy Title
AHS-G2036	Hepatitis Testing
AHS-G2110	Serum Marker Panels for Hepatic Fibrosis in the Evaluation and Monitoring of Chronic Liver Disease

Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

This policy is specific to individuals 18 years of age or older. Criteria below do not apply to individuals less than 18 years of age.

1) For individuals with elevated alkaline phosphatase activity, serum GGT testing no more than once every two weeks **MEETS COVERAGE CRITERIA**.

2) To assess for liver injury, function, and/or disease, serum GGT testing no more than once every two weeks **MEETS COVERAGE CRITERIA** for individuals with at least one of the following conditions:
   a) For individuals with chronic alcohol use.
   b) For individuals on a long-term drug therapy known to have a potential for causing liver toxicity.
   c) For individuals with exposure to hepatotoxins.
   d) For individuals with viral hepatitis, amoebiasis, tuberculosis, psittacosis, or similar infections that may cause hepatic injury.
   e) For individuals with primary or secondary malignant neoplasms.
   f) For individuals with diabetes mellitus.
   g) For individuals with malnutrition.
   h) For individuals with disorders of iron and mineral metabolism.
   i) For individuals with sarcoidosis.
   j) For individuals with amyloidosis.
   k) For individuals with lupus.
   l) For individuals with hypertension.
   m) For individuals with gastrointestinal disease.
   n) For individuals with pancreatic disease.
   o) To assess liver function subsequent to liver transplantation.

3) For asymptomatic individuals, serum GGT testing during a wellness visit or a general exam without abnormal findings **DOES NOT MEET COVERAGE CRITERIA**.

Table of Terminology

Term	Definition
AACC	American Association of Clinical Chemistry
ACG	American College of Gastroenterology
AF	Atrial fibrillation
ALEH	American Association for the Study of the Liver

ALP	Alkaline phosphatase
ALT	Aminotransferase
ANCA	Anti-Neutrophilic Cytoplasmic Autoantibody
AP	Alkaline phosphatase
APRI	Aspartate aminotransferase-to-platelet ratio index
ASAM	American Society of Addiction Medicine
AST	Aminotransferase
ASV	Average successive variability
BSG	British Society of Gastroenterology
CAG	Canadian Association of Gastroenterology
CAGE	Cut, Annoyed, Guilty, and Eye-Opener
CBC	Complete blood count
CDT	Carbohydrate-deficient transferrin
CHD	Coronary heart disease
CKD	Chronic kidney disease
CLIA ’88	Clinical laboratory improvement amendments of 1988
CMS	Centers for Medicare and Medicaid Services
CSSC	Clinical Services and Standards Committee
CVA	Cerebrovascular accident
CVD	Cardiovascular disease
DB	Direct bilirubin
DILI	Drug-induced liver injuries
EASL	European Association for Study of Liver
FBC	Full blood count
FDA	Food and Drug Administration
FIB-4	Fibrosis-4
GGT	Gamma-glutamyl transferase
GGTP	Gamma glutamyl transpeptidase
HDL-C	High-density lipoprotein cholesterol
HIBD	High-intensity binge drinking
INR	International normalized ratio
KIM-1	Kidney injury molecule-1
LDT	Laboratory-developed test
LFT	Liver function test
MCV	Mean corpuscular volume
Mets	Metabolic syndrome
MI	Myocardial infarction
MR	Mendelian randomization
NAFLD	Non-alcoholic fatty liver disease
NCD	National coverage determination
NSAIDs	Nonsteroidal anti-inflammatory drugs

PT	Prothrombin time
TBL	Total bilirubin
TC	Total cholesterol
TE	Transient elastography
TRG	Triglycerides

Scientific Background

Gamma-glutamyl transferase (GGT) is a cell surface enzyme found throughout the body. GGT cleaves extracellular glutathione (an antioxidant) and other gamma-glutamyl compounds to increase the availability of amino acids for intracellular glutathione synthesis purposes. GGT also plays an important role in maintaining glutathione homeostasis, as well as in providing defense against oxidative stress (Ndrepepa & Kastrati, 2016). The measurement of circulating GGT is often used as a diagnostic tool for the identification of liver diseases, biliary diseases, and alcohol consumption. This is because GGT is very abundant in the liver; considerable GGT concentrations are also found in the intestine, kidney, prostate, and pancreas (Newsome et al., 2018). While GGT measurement may not be useful in the diagnosis of specific types of liver disease, it is one of the best predictors of overall liver mortality (Newsome et al., 2018). Additional research has shown that elevated GGT concentrations in the serum may also be associated with an increased risk of type 2 diabetes, gestational diabetes, hypertension, stroke, coronary heart disease, and cancer (Koenig & Seneff, 2015). Abnormal GGT levels are also identified in anorexia nervosa, Guillain-Barré syndrome, hyperthyroidism, obesity, dystrophica myotonica (Gowda et al., 2009), and cigarette smoking (AACC, 2024). Certain drugs may lead to unusual GGT levels in the blood as well. It has been reported by the AACC (2024) that drugs such as phenytoin, carbamazepine, barbiturates (including phenobarbital), lipid-lowering drugs, antibiotics, antifungal agents, anticoagulants, immunosuppressive medications, antidepressants, hormones, nonsteroidal anti-inflammatory drugs (NSAIDs), oral contraceptives, testosterone, and histamine receptor blockers may cause an increase or decrease in GGT levels. LabCorp (2021) does not recommend ordering a GGT test if the patient is currently taking phenytoin or phenobarbital since these medications may lead to false elevations in GGT.

Gamma-glutamyl transferase measurement may also be a useful secondary measure to assist with liver diagnoses. Alkaline phosphatase (ALP) is an enzyme found throughout the body and is typically identified in the liver or bone. Meanwhile, GGT is not found in bone (Singh et al., 2006). Therefore, if elevated ALP levels are detected in a patient, physicians may use a high GGT level to rule out bone disease as the cause of an elevation of ALP; however, if GGT is low or normal, then elevated ALP levels are more likely to be caused by bone disease (AACC, 2024). This means that elevated GGT levels suggest that elevated ALP levels are of a hepatic origin (Kwo et al., 2017).

Koenig and Seneff (2015) report that population wide GGT levels have increased steadily in the United States over the last three decades. This may factor into an increased disease risk over time. It has been hypothesized that GGT levels are increasing due to a greater exposure to environmental and endogenous toxins which result in increased levels of oxidative and nitrosative stress (Koenig & Seneff, 2015). Elevated serum GGT levels are known markers of oxidative stress, which occurs when an imbalance is present between antioxidants and free radicals in the body (Yamada et al., 2006). Simple lifestyle changes, such as avoiding exposure to toxic chemicals and limiting iron intake, may help to lower GGT levels.

Liver function tests are blood tests typically ordered as a panel rather than solitarily. These tests measure the level of several liver enzymes in serum or plasma samples. The liver enzymes frequently measured to

detect liver abnormalities include serum alanine aminotransferase (Nivukoski et al.), aspartate aminotransferase (AST), ALP, and bilirubin; other liver tests may incorporate the measurement of GGT, albumin and prothrombin time (Kwo et al., 2017). Some report that GGT is only occasionally included in a liver function testing panel (Dillon & Miller, 2016), while others report that GGT is still a commonly measured serum liver enzyme (Friedman, 2024). Nevertheless, Dillon and Miller (2016) conclude that GGT should be measured on liver functioning test panels "some of the time". This is likely because GGT measurement is not very specific, and its elevation will typically not help the physician to differentiate between diseases.

GGT and Liver-Related Diseases

The liver is an organ in the abdomen which detoxifies metabolites, manufactures proteins, and generates biochemicals required for growth and digestion. Many types of liver disease exist, such as hepatitis A, hepatitis B, hepatitis C, cirrhosis, fatty liver disease, and liver cancer to name a few. GGT is elevated in the blood in most diseases that cause damage to the liver, including hepatitis and cirrhosis (AACC, 2024). Primary biliary cholangitis (PBC), drug-induced liver injury (DILI), alcoholic liver disease (ALD), and non-alcoholic fatty liver disease (NAFLD) are the main causes of the abnormal GGT in clinic. GGT levels have different characteristics in different liver diseases. For instance, abnormal GGT in PBC and DILI was associated with cholestasis; in ALD, it was associated with both oxidative stress and cholestasis, and in NAFLD, it was associated with oxidative stress (Xing et al., 2022).

Hepatitis C is a viral infection that targets the liver and causes inflammation. An increase in serum GGT levels is seen in approximately 30% of patients with a chronic hepatitis C infection; GGT levels will peak in the second or third week of illness and may remain elevated for up to six weeks (Gowda et al., 2009). Further, the GGT-to-platelet ratio has been identified as a reliable laboratory marker in the prediction of liver fibrosis stage in patients with a chronic hepatitis B infection; this ratio was more reliable than AST-to-platelet ratio index (Vos et al.) and fibrosis-4 score (FIB-4) (Lee et al., 2018; Wang et al., 2016). The FIB-4 score is a non-invasive scoring system based on several laboratory tests to estimate the amount of scarring in the liver. GGT is also acknowledged as a more specific tool for the identification of non-alcoholic fatty liver disease than ALT (Dillon & Miller, 2016). Finally, GGT has also been identified as a useful prognostic tool for patients with hepatocellular carcinoma, the most common type of primary liver cancer (Wang et al., 2014).

Lothar (2022) notes that acute viral hepatitis A and B are usually self-limiting and that almost all cases of hepatitis A and 95% of cases of hepatitis B are cured. In contrast, about 85% of acute hepatitis C infections proceed into a chronic form. "During the acute phase of hepatitis, the aminotransferases do not allow any conclusion as to whether hepatitis will be cured or develop into a chronic form in individual cases. The ALT and GGT are the last enzymes to return to normal levels. Monitoring is recommended including measurements every 2 weeks. If the enzyme levels have not normalized within 6 months or show recurrent elevations, a chronic form must be expected. This always applies if no antibodies against HBsAg and HBeAg are produced or if virus persistence is detected" (Lothar, 2022).

GGT and Bile Duct Diseases

The bile ducts are thin tubes that connect the liver to the small intestine. These ducts help to transport bile from the liver and gallbladder to the small intestine; the bile then assists with the digestion of fats in foods. Singh et al. (2006) report that in 55 patients aged 23 to 45 years, "GGT and ALP levels were normal in patients of chronic cholecystitis with cholelithiasis but significantly high in patients of common bile duct obstruction".

GGT and Kidney/Renal Diseases

The kidneys filter the body's blood by removing waste and maintaining electrolyte balance. Acute kidney or renal injuries are sudden episodes of kidney damage or failure. Lippi et al. (2018) showed that, in dogs with acute kidney injury, significantly higher GGT urine levels were identified.

Chronic kidney disease (CKD) occurs when the kidneys are no longer able to filter blood correctly. Several liver enzyme serum levels, including GGT, have been measured in patients with CKD. However, one analysis reported that relevant GGT data were scant and that "those found reported that there were no differences between the patients with or without chronic kidney disease" (Sette & Almeida Lopes, 2014). Noborisaka et al. (2013) researched elevated serum GGT levels in cigarette smokers and monitored the development of CKD. The authors completed a 6-year retrospective study on 2,603 male workers and concluded that the "elevation of serum GGT in smokers, to a large extent, depends on the associated alcohol consumption. Elevated GGT in smokers plays at least a partial role in the development of CKD, mainly proteinuria, and the underlying mechanisms remain to be elucidated" (Noborisaka et al., 2013).

In another study, the authors claimed that GGT variability may be able to predict the risk of end-stage renal disease (ESRD). GGT variability was assessed using the average successive variability, standard deviation, and CV of serial measurements of GGT during the five years before the baseline examination. Subjects were divided into four quartiles and those in GGT ASV quartile four were older, more obese, and had higher BP and more comorbidities than those in quartile one. The metabolic variables got worse as the baseline GGT quartile increased. Overall, the implications of GGT levels were statistically significant, especially in women and in ESRD caused by diabetic nephropathy (Lee et al., 2020).

GGT and Pancreatic Diseases

The pancreas is in the abdomen and helps to regulate blood sugar and digestion. Several disorders of the pancreas exist, including type 1 diabetes, type 2 diabetes, pancreatic cancer, and pancreatitis. Elevated GGT levels have been used as a prognostic factor to predict survival time in patients with unresectable pancreatic cancer (Engelken et al., 2003).

Pancreatitis occurs when the pancreas becomes inflamed due to its own digestive chemicals. Elevated GGT levels are often identified in patients with acute and chronic pancreatitis (Vroon & Israili, 1990). However, Gori et al. (2019) recently researched the GGT to urinary creatinine ratio in dogs with acute pancreatitis and found no association with any outcome in the study.

GGT and Alcohol Consumption

Increased levels of GGT and alcohol consumption are often correlated. Still, this relationship varies between individuals. GGT concentrations may increase with only small amounts of alcohol consumption in some; on the other hand, only about 75% of chronic drinkers will have elevated GGT levels (AACC, 2024). GGT assays have been widely used as an "index of liver dysfunction and marker of alcohol intake. The half-life of GGT is between 14 and 26 days and after stopping drink it returns to normal level in 4-5 weeks" (Dixit & Singh, 2015). Nivukoski et al. (2019) report that regular alcohol use is associated with increased GGT and ALT levels. Choe et al. (2019) report that GGT has low sensitivity as a blood biochemical marker of excessive alcohol intake, but the combined use of the CAGE questionnaire (a four-question questionnaire widely used to screen for alcohol problems) and the measurement of serum GGT is a useful tool for alcohol dependence screening.

GGT and Metabolic Syndrome-Related Risk

Metabolic syndromes are a group of conditions which include high blood sugar, high blood pressure (hypertension), obesity, and abnormal cholesterol levels. GGT has been identified as a biomarker for metabolic syndrome risk (Grundy, 2007). Further, Lee et al. (2019) report that GGT levels are significantly higher in subjects with a metabolic syndrome-related disorder than in healthy individuals. Metabolic syndromes collectively increase an individual's risk for the development of many diseases, including heart disease, stroke, type 2 diabetes, and neurologic disorders.

Cardiovascular Disease

Cardiovascular disease (CVD), also known as heart disease, encompasses a group of conditions that narrow or block a blood vessel. This may lead to a heart attack, chest pain or stroke. Ndrepepa and Kastrati (2016) previously stated that while more research needs to be conducted, "Ample evidence suggests that elevated GGT activity is associated with increased risk of CVD such as coronary heart disease (CHD), stroke, arterial hypertension, heart failure, cardiac arrhythmias, and all-cause and CVD-related mortality. The evidence is weaker for an association between elevated GGT activity and acute ischemic events and myocardial infarction". GGT has been widely identified as a biomarker for cardiovascular risk; in particular, high levels of GGT are associated with a greater risk of atherosclerotic cardiovascular disease (Grundy, 2007), and high GGT variability is associated with an increased risk of myocardial infarction and CVD related mortality (Chung et al., 2019). GGT and the risk of atherosclerosis and coronary heart disease has been reported by Ndrepepa, Colleran, et al. (2018) who report that "it remains unknown whether GGT plays a direct role in the pathophysiology of atherosclerosis and CHD or is merely a correlate of coexisting cardiovascular risk factors". A study by Arasteh et al. (2018) researched how serum GGT can be used as a predictive biomarker for stenosis severity in patients with coronary artery disease; these authors report a significant association between serum GGT activity and patients with coronary artery disease. GGT is considered an inexpensive and readily available biomarker that may provide more information than current tools on the prediction of coronary plaque burdens and plaque structures in young adults (Celik et al., 2014).

Cerebrovascular Accident

A cerebrovascular accident (CVA) or stroke occurs when a blood vessel leading to the brain ruptures or is blocked by a blood clot. There are three main types of CVAs: transient ischemic attack, ischemic stroke, and hemorrhagic stroke. A transient ischemic attack only lasts a few minutes and occurs because of a temporary blood vessel blockage to part of the brain. An ischemic stroke occurs when an artery in the brain is completely blocked, and a hemorrhagic stroke occurs when a ruptured blood vessel causes bleeding in the brain. Several studies have identified a relationship between GGT levels and both hemorrhagic and ischemic CVAs (Korantzopoulos et al., 2009; Xu et al., 2017; Yao et al., 2019).

Gamma-glutamyl transferase levels have been associated with functional outcomes after an aneurysm and/or stroke. Xu et al. (2017) state that patients with high GGT levels are more likely to have a poor prognosis after aneurysmal subarachnoid hemorrhage than patients with lower GGT levels, suggesting that serum GGT may be an important prognostic factor for the prediction of aneurysm outcomes. Yang et al. (2020) also report that high GGT levels were significantly associated with cardioembolic stroke through atrial fibrillation (irregular heartbeat). More, GGT variability has been associated with an increased risk of stroke in the general population (Chung et al., 2019), and serum GGT levels have been associated with a greater risk of ischemic or nonembolic stroke in individuals older than 70 years (Korantzopoulos et al., 2009). Serum GGT levels were also found to be significantly elevated in patients

who died from an acute ischemic stroke, and high GGT levels were associated with an increased risk of death in male patients with an intracranial arterial calcification (Yao et al., 2019).

Type 2 Diabetes

Type 2 diabetes occurs when the body either does not produce enough insulin or resists insulin. Diabetes and GGT levels have been researched by Kaneko et al. (2019) who state that the simultaneous elevation of GGT and ALT is significantly associated with the development of type 2 diabetes mellitus; confounding factors include alcohol consumption and obesity. Further, when GGT and ALT were included in type 2 diabetes risk prediction, the accuracy of the prediction was improved (Kaneko et al., 2019). Kunutsor et al. (2014) report that greater circulating GGT levels lead to an increased risk of type 2 diabetes. Higher GGT levels have also been associated with a greater amount of insulin resistance and therefore a higher risk of developing the disease (Grundy, 2007).

Nano et al. (2017) analyzed 1125 cases of prediabetes and 811 cases of type 2 diabetes. A mendelian randomization (MR) study was performed and the authors found that "MR analyses did not support a causal role of GGT on the risk of prediabetes or diabetes. The association of GGT with diabetes in observational studies is likely to be driven by reverse causation or confounding bias. As such, therapeutics targeted at lowering GGT levels are unlikely to be effective in preventing diabetes" (Nano et al., 2017). This study is important as the results contradict other related studies. Another bidirectional mendelian randomization study analyzed data from 64,094 individuals with type 2 diabetes and 607,012 control subjects; no association between GGT and type 2 diabetes risk was found (De Silva et al., 2019). Further, Shibabaw et al. (2019) also report that, based on their study, GGT levels were not significantly higher in type 2 diabetes patients compared to healthy controls (P=0.065).

Neurodegenerative Diseases

Abnormal GGT serum levels have been associated with an increased risk of neurodegenerative disease development. The serum GGT levels and Parkinson disease risk in men and women analyzed by Yoo et al. (2020) suggest that the top quartile of patients with high serum GGT levels was associated with a lower Parkinson disease risk in men and a higher risk in women (n=20,895 Parkinson disease patients). Another study focused on Alzheimer disease showed that alcohol consumption was associated with an earlier Alzheimer disease age of onset survival and increased GGT blood concentration levels (Andrews et al., 2020). Alcohol consumption and GGT levels were not associated with late onset Alzheimer disease risk. Further, Hong et al. (2020) recently reported that GGT variability may lead to an increased risk of all-cause dementia, and Yavuz et al. (2008) found that GGT levels were increased significantly in Alzheimer disease patients in a cross-sectional study of 132 patients with Alzheimer disease and 158 healthy age-matched controls.

Clinical Utility and Validity

Individuals infected with hepatitis C virus are at an increased risk of developing hepatocellular carcinoma even after a sustained virological response is achieved. A total of 642 patients who had achieved a sustained virological response after a hepatitis C infection participated in this study; 33 participants developed hepatocellular carcinoma (Huang et al., 2014). The data showed that "Baseline gamma-glutamyl transferase [GGT] levels strongly correlate with hepatocellular carcinoma development in non-cirrhotic patients with successful hepatitis C virus eradication," suggesting that serum GGT measurement may help to identify specific patients at high risk for developing hepatocellular carcinoma (Huang et al., 2014).

The relationship between liver enzymes and the risk of metabolic syndrome have been researched several times. Liu et al. (2018) completed a large cross-sectional study with 1444 elderly participants to determine the association between liver enzymes and the risk of metabolic syndrome. The authors noted that "The prevalence of MetS [metabolic syndrome] and its components increased remarkably with increasing quartiles of alanine aminotransferase (Nivukoski et al.), gamma-glutamyltransferase (GGT) and alkaline phosphatase (ALP) but not with aspartate aminotransferase (AST) in the elderly," showing that these liver enzymes as positively associated with metabolic syndrome development in elderly populations (Liu et al., 2018). Another study completed by Wang et al. (2017) assessed liver function and metabolic syndrome. This study enrolled 32,768 ostensibly healthy participants. Regarding GGT, the authors note that the metabolic syndrome risk "significantly increased … in high quartiles for both genders," suggesting that high GGT levels are a risk factor for the development of metabolic syndromes (Wang et al., 2017).

Ndrepepa, Holdenrieder, et al. (2018) compared GGT and ALP to see which was a better prognostic marker for mortality in patients with coronary heart disease. A total of 3768 patients with coronary heart disease participated in this three year study. The median value of GGT was 36.2 U/L and the median value of ALP was 69.3 U/L; "Overall, there were 304 deaths: 195 deaths occurred in patients with GGT >median (n = 1882) and 109 deaths occurred in patients with GGT ≤median (n = 1886) … According to ALP activity, 186 deaths occurred in patients with ALP >median (n = 1883) and 118 deaths occurred in patients with ALP ≤median (n = 1885)" (Ndrepepa, Holdenrieder, et al., 2018). The authors conclude that GGT is a stronger prognostic marker for all-cause mortality in patients with coronary heart disease than ALP.

Conigrave et al. (2002) completed a large, multicenter study with 1863 participants from five countries. This study aimed to measure carbohydrate-deficient transferrin (CDT) and GGT as markers of alcohol consumption. The authors concluded that "CDT was [a] little better than GGT in detecting high- or intermediate-risk alcohol consumption in this large, multicenter, predominantly community-based sample. As the two tests are relatively independent of each other, their combination is likely to provide better performance than either test alone. Test interpretation should take account sex, age, and body mass index" (Conigrave et al., 2002).

Rosoff et al. (2019) studied the association between lipid and liver function enzymes and high-intensity binge drinking (HIBD). This cross-sectional study included 1519 participants. Binge drinking was defined according to the National Institute on Alcohol Abuse and Alcoholism. GGT was one of several enzymes measured (others included high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol, total cholesterol (Newsome et al.), triglycerides (TRG), ALT and AST). The authors state that "HIBD was associated with increased levels of HDL-C, TC, TRG, ALT, AST, and GGT" (Rosoff et al., 2019). Further, the authors also note that the largest increases associated with HIBD was found based on GGT levels, suggesting that "GGT may be most sensitive to HIBD" (Rosoff et al., 2019).

A study completed by Jousilahti et al. (2000) researched the relationship between serum GGT levels, self-reported alcohol consumption and the risk of stroke. A total of 14,874 participants took part in this study over five years. The authors report that "serum GGT concentration was associated with the risk of total and ischemic stroke in both genders. There was also a significant association among men between GGT and the risk of intracerebral hemorrhage and among women between GGT and the risk of subarachnoid hemorrhage" (Jousilahti et al., 2000). Further, a relationship was not found regarding self-reported alcohol and any type of stroke.

Yang et al. (2020) studied the effects of GGT on stroke occurrence mediated by atrial fibrillation (AF). A total of 880 patients with acute ischemic stroke participated in this study, and AF was identified in 132 of the patients. The authors found that high GGT levels were not associated with large-artery atherosclerosis stroke but were associated with cardioembolic stroke. "The GGT level was significantly associated with cardioembolic stroke via AF. The results obtained in the present study may explain why GGT is associated with stroke" (Yang et al., 2020).

Hong et al. (2020) completed a study to determine if there was a relationship between GGT variability and dementia risk in diabetes mellitus patients. This study included 37,983 diabetic patients who were diagnosed with dementia over a 6.12-year follow-up period. "In the fully adjusted model, the group with the highest quartile of GGT variability had a 19% increased risk of all-cause dementia when compared with the lowest quartile group" (Hong et al., 2020). The authors conclude by stating that in patients with diabetes mellitus, a high amount of GGT variability increased the risk of dementia regardless of other factors such as baseline GGT level.

Lee et al. (2020) examined the prognostic value of GGT variability in predicting the risk of stroke, myocardial infarction, and mortality in diabetic patients. A total of 698,937 patients greater than 40 years of age, with a history of diabetes, and without a history of stroke, MI, liver cirrhosis, or chronic hepatitis were included in the study. GGT variability was assessed as the average successive variability (ASV) of serial GGT measurements during the five years before the baseline examination. Subjects were stratified according to quartiles of baseline GGT and GGT ASV. The lower quartile contained subjects with lower GGT levels. According to the results, subjects in GGT ASV quartile four were more obese were more likely to have hypertension, dyslipidemia, or chronic kidney disease, and had a higher risk for stroke, MI, and mortality. On the other hand, subjects in quartile one were older, and had a higher prevalence of chronic kidney disease but a lower prevalence of hypertension and obesity. The authors conclude that GGT variability is associated with a higher risk of stroke, MI, and mortality; therefore, "it is important to identify the factors that contribute to increased GGT variability to extend the lives of patients with diabetes" (Lee et al., 2020).

Mujawar et al. (2020) studied the use of salivary gamma-glutamyl transpeptidase as a biomarker in oral squamous cell carcinoma and precancerous lesions. Seventy-five patients with precancerous lesions or oral squamous cell carcinoma were enrolled in the study and assessed for GGT levels. Healthy participants had a GGT between 4 to 30U/L, those with precancerous lesions had GGT between 39 to 65 U/L, and those with oral squamous cell carcinoma had GGT levels between 53 and 86 U/L. The authors conclude that it can be a reliable biomolecular marker in early detection and prevention of oral cancer that could be routinely employed in dental clinics (Mujawar et al., 2020).

Li et al. (2022) studied the association of GTT levels with the occurrence of post-stroke cognitive impairment (PSCI). A total of 1,957 participants with a minor ischemic stroke or transient ischemic attack were measured for GTT and they were categorized into four quartiles based on baseline GTT levels. Of the 1,957 participants, 671 (34.29%) patients experienced PSCI at three months of follow-up. The highest GGT level quartile group exhibited a lower risk of PSCI. The authors conclude that "serum GGT levels are inversely associated with the risk of PSCI, with extremely low levels being viable risk factors for PSCI" (Li et al., 2022).

Guidelines and Recommendations

American College of Gastroenterology (ACG)

Guidelines from the ACG recommend the following:

- "Before initiation of evaluation of abnormal liver chemistries, one should repeat the lab panel and/or perform a clarifying test (e.g., GGT if serum alkaline phosphate is elevated) to confirm that the liver chemistry is actually abnormal. (Strong recommendation, very low level of evidence).
- An elevation of alkaline phosphatase should be confirmed with an elevation in GGT. Given its lack of specificity for liver disease, GGT should not be used as a screening test for underlying liver disease in the absence of other abnormal liver chemistries. (Strong recommendation, very low level of evidence).
- An elevated alkaline phosphatase level of hepatic origin may be confirmed by elevation of gamma-glutamyl transferase (GGT) or fractionation of alkaline phosphatase
- Measurement of GGT may represent a complementary test to identify patterns of alcoholism or alcohol abuse, although GGT by itself is not helpful in establishing a diagnosis of alcoholic liver disease
- If the alkaline phosphatase is elevated in the presence of other elevated liver chemistries, confirmation of hepatic origin is not required. With isolated alkaline phosphatase elevation, confirmation with GGT, or fractionation of alkaline phosphatase isoenzymes can be used to help differentiate liver alkaline phosphatase from non-liver sources. However, GGT elevation is not specific for cholestatic liver disease, and can be elevated in >50% of alcoholic patients without obvious evidence of liver disease. GGT can also be elevated in patients with pancreatic disease, myocardial infarction, renal failure, emphysema, diabetes, and in patients taking certain medications such as phenytoin and barbiturates. Given its lack of specificity for liver disease, GGT should not be used as a screening test for underlying liver disease in the absence of abnormal liver chemistries
- Those who present with an elevation in alkaline phosphatase with normal AST, ALT, and bilirubin levels should have their alkaline phosphatase elevation confirmed with a GGT level and if elevated an ultrasound of the liver should be ordered" (Kwo et al., 2017).

European Association for Study of Liver (EASL)

The EASL published clinical practice guidelines for drug-induced liver injuries (DILI). These guidelines state that "ALT, ALP and TBL [total bilirubin] are the standard analytes to define liver damage and liver dysfunction in DILI. AST [aspartate aminotransferase] values can be used to reliably substitute ALT in calculating the pattern of injury when the latter is unavailable at DILI recognition, whereas GGT is less reliable as an ALP substitute. Grade C" (Andrade et al., 2019).

The EASL also published clinical practice guidelines for the management of alcohol-related liver disease (ALD). These guidelines state that "As the measurement of GGT, ALT, AST and MCV [mean corpuscular volume] is easy and inexpensive, they remain the most frequently used markers for early detection of ALD. However, all these laboratory values are only indirect markers for ALD, with low sensitivity and specificity... No single marker or combination of markers can differentiate between different causes of liver disease" (Thursz et al., 2018). The authors also note that "Screening investigations should not only include liver function tests (LFTs), i.e. gamma glutamyl transpeptidase (GGT[P]), serum ALT and serum AST, but also performance of a test to detect liver fibrosis (e.g. TE [transient elastography])" (Thursz et al., 2018).

In a 2021 update, the EASL asserted that "In patients with elevated liver stiffness and biochemical evidence of hepatic inflammation (AST or GGT >2xULN), LSM by TE should be repeated after at least 1

week of alcohol abstinence or reduced drinking **(LoE 3; strong recommendation)**" (Berzigotti et al., 2021).

European Association for Study of Liver (EASL) and Latin American Association for the Study of the Liver (ALEH)

Guidelines from the EASL and ALEH state that "Serum biomarkers can be used in clinical practice due to their high applicability (>95%) and good interlaboratory reproducibility. However, they should be preferably obtained in fasting patients (particularly those including hyaluronic acid) and following the manufacturer's recommendations for the patented tests" (Castera et al., 2015). The guidelines provide a list of several serum biomarkers including GGT.

Canadian Association of Gastroenterology (CAG)

The CAG practice guidelines for the evaluation of abnormal liver enzyme tests state that GGT may be used as a second-line biochemical test. Specifically, the guidelines state that "All patients with at least one abnormal liver screening test (abnormal ALT, AST or ALP) should have the following liver biochemical tests performed: gamma-glutamyl transferase (GGT), albumin, bilirubin (including direct if the total bilirubin is elevated) and either prothrombin time (PT) or international normalized ratio (INR). These tests can be performed as initial screening tests if it is inconvenient for the patient to return to the physician's office within a reasonable period of time (weeks or months depending on the severity of the enzyme abnormalities)" (Minuk, 1998).

American Society of Addiction Medicine (ASAM)

The ASAM released clinical practice guidelines on the use of laboratory tests which measure impairment of hepatic functioning. ASAM recommends measurement of GGT and ALT to identify recent heavy alcohol use and risk for alcohol withdrawal, and notes that when conducting a urine test, GGT is recommended as the marker of heavy alcohol consumption (ASAM, 2020).

Clinical Services and Standards Committee (CSSC) of the British Society of Gastroenterology (BSG)

The Clinical Services and Standards Committee of the British Society of Gastroenterology was commissioned to produce guidelines for the management of abnormal liver blood tests. They recommend that the "Initial investigation for potential liver disease should include bilirubin, albumin, ALT, ALP and GGT, together with a full blood count if not already performed within the previous 12 months. (level 2b, grade B)". They note that "If there is clear indication of a specific clinical risk—for example, in high-risk groups such as injecting drug users, migrants from high prevalence areas or prisoners, then some aspects of second-line testing can be undertaken simultaneously. In many patients with liver damage an assessment of liver fibrosis is critical in making decisions about referral and management." They go further to explain that in adults, "clues to the level of liver fibrosis can be gleaned from the use of non-invasive algorithms such as the AST to ALT ratio" such that an AST:ALT greater than one indicated advanced fibrosis or cirrhosis, but warns that "non-invasive markers have not been sufficiently validated in children to be routinely applied in clinical practice" (Newsome et al., 2018).

Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
82977	Glutamyltransferase, gamma (GGT)

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

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Revision History

Revision Date	Summary of Changes
09/04/2024	Reviewed and Updated: Updated the background, guidelines and recommendations, and evidence-based scientific references. Literature review did not necessitate any modifications to coverage criteria. The following changes were made for clarity and consistency: Coverage is limited to GGT testing in adults. For clarity, this results in a title change from "Gamma-glutamyl Transferase" to "Gamma-glutamyl Transferase Testing in Adults" As this policy is specific to adults (individuals 18+), a disclaimer was added to Section III and CC1 and 2 were simplified to remove references to age restrictions. The beginning of Section III now reads: "This policy is specific to individuals 18 years of age or older. Criteria below do not apply to individuals less than 18 years of age.

	1) For individuals with elevated alkaline phosphatase activity, serum GGT testing no more than once every two weeks MEETS COVERAGE CRITERIA. 2) To assess for liver injury, function, and/or disease, serum GGT testing no more than once every two weeks MEETS COVERAGE CRITERIA for individuals with at least one of the following conditions:"

General Inflammation Testing

Policy Number: AHS – G2155 – General Inflammation Testing	Prior Policy Name and Number, as applicable:
Initial Presentation Date: 07/26/2018 Effective Date: February 1, 2025	

Policy Description

Inflammatory response can occur due to tissue injury and/or various disorders, including arthritis, lupus, and infection. Acute phase reactants, such as serum C-reactive protein (CRP), are released in the acute phase response during inflammation and can be used to monitor inflammation. Inflammation may also be measured using the simple laboratory technique of erythrocyte sedimentation rate (ESR) (Kushner, 2024).

For guidance on the use of CRP as a cardiac biomarker, please see policy AHS-G2150-Biomarkers for Myocardial Infarction and Chronic Heart Failure. For guidance on the use of CRP as a marker for acute pancreatitis, please see AHS-G2153-Pancreatic Enzyme Testing for Acute Pancreatitis.

Related Policies

Policy Number	Policy Title
AHS-G2150	Biomarkers for Myocardial Infarction and Chronic Heart Failure
AHS-G2153	Pancreatic Enzyme Testing for Acute Pancreatitis

Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

i. Measurement of C-reactive protein (CRP) and/or erythrocyte sedimentation rate (ESR) **MEETS COVERAGE CRITERIA** for inflammatory conditions as specified in Note 1.

ii. For individuals without a diagnosed inflammatory condition, measurement of ESR **DOES NOT MEET COVERAGE CRITERIA.**

iii. Measurement of CRP and/or ESR during general exam without abnormal findings **DOES NOT MEET COVERAGE CRITERIA**.

NOTES:

Note 1: Coverage of CRP, ESR, CRP or ESR, or both CRP and ESR is designated based on the diagnosed or suspected inflammatory condition. Either conventional or high-sensitivity CRP testing are allowed methods of testing for CRP levels. When either CRP **or** ESR are allowed, CRP is the preferred biomarker.

Condition	Test Preference	Frequency of Testing
Acute and Chronic Urticaria	CRP or ESR	Not specified (NS)
Acute Hematogenous Osteomyelitis (AHO)	CRP	To confirm diagnosis; 2 to 3 days during the early therapeutic course; weekly until normalization (or a clear trend toward normalization is evident)
Acute Phase Inflammation	CRP	NS
Ankylosing Spondylitis	CRP or ESR	Regular interval use in patients with active symptoms
Arthritis	CRP and ESR	1-3 months initially; 6-12 months later
Castleman's Disease	CRP	NS
General Inflammation	CRP	NS
Hodgkin Lymphoma	ESR	Every 3 to 6 months for 1 to 2 years; every 6 to 12 months for the next 3 years; annually thereafter
Irritable Bowel Syndrome	CRP and ESR	During initial assessment to exclude other diagnoses
Large Vessel Vasculitis (Giant Cell Arteritis, Takayasu Arteritis)	CRP and ESR	To confirm diagnosis; every 1–3 months during the first year; every 3–6 months thereafter

Nonradiographic axial spondyloarthritis	CRP or ESR	Regular interval use in patients with active symptoms
Polymyalgia Rheumatica	CRP or ESR	At initial diagnosis; every 3 months during long-term steroid therapy
Periprosthetic Joint Infections (PJI)	CRP and ESR	NS
Rheumatoid Arthritis	CRP or ESR	Prior to treatment; every 1-3 months during active disease; annually when disease is inactive
Systemic Lupus Erythematosus	CRP or ESR	At initial assessment; every 1-3 months during active disease; every 6-12 months during stable disease; during pregnancy
T-cell lymphomas	ESR	NS

Table of Terminology

Term	Definition
AAAAI	Academy of Allergy, Asthma & Immunology
AAFP	American Academy of Family Physicians
AAOS	American Association of Orthopaedic Surgeons
AAOS	American Academy of Orthopaedic Surgeons
ABIM	American Board of Internal Medicine
ABVD	Adriamycin, bleomycin, vinblastine, dacarbazine
ACAAI	American College of Allergy, Asthma & Immunology
aCL	Anticardiolipin
ACPA	Anti-cyclic citrullinated peptide antibodies
ACR	American College of Rheumatology
ACR	American College of Radiology
ANA	Antinuclear antibodies
Anti-CCP	Anti-cyclic citrullinated peptides
Anti-dsDNA	Anti-double stranded DNA
Anti-β2-GPI	Anti-β2-glycoprotein I
aPL	Antiphospholipid antibodies
AS	Ankylosing spondylitis
ASCP	American Society for Clinical Pathology
ASCP	Anti-cyclic citrullinated peptide antibodies
AUC	Area under the curve
BHPR	British Health Professionals in Rheumatology
BSR	British Society for Rheumatology

CBC	Complete blood count
cCRP	Cardiac C-reactive protein
CDAI	Clinical disease activity index
CHL	Classic Hodgkin lymphoma
CLIA	Clinical laboratory improvement act
CRA	Canadian Rheumatology Association
CRP	C-reactive protein
CTD	Connective tissue diseases
CVD	Cardiovascular disease
DAS	Disease activity score
DAS28	28-Joint disease activity score
DAS28-CRP	Disease activity score 28 C-reactive protein
DAS28-ESR	Disease activity score with 28-joint counts - erythrocyte sedimentation rate
EDL	Essential In Vitro Diagnostics
EDTA	Ethylenediamine tetraacetic acid
eGFR	Estimated glomerular filtration rate
EIA	Enzyme immunoassay
ENA	Extractable nuclear antigens
ESR	Erythrocyte sedimentation rate
EULAR	European League Against Rheumatism
FDA	Food and Drug Administration
GCA	Giant cell arteritis
HCSC	Health care service corporation
HL	Hodgkin lymphoma
hsCRP	High-sensitivity C-reactive protein
IBD	Inflammatory bowel disease
IBS	Irritable bowel syndrome
ICSH	International Council for Standardization in Hematology
ISRT	Involved-site radiation therapy
IVD	In vitro diagnostics
JTFPP	Joint Task Force on Practice Parameters
LAC	Lupus anticoagulant
LDH	Lactate dehydrogenase
MCD	Multicentric Castleman Disease
MSIS	Musculoskeletal Infection Society
NA	Not applicable
NASH	Nonalcoholic steatohepatitis
NCCN	National Comprehensive Cancer Network
NICE	National Institute for Health and Care Excellence
NPV	Negative predictive value
NS	Not specified

NSAID	Non-steroidal anti-inflammatory drugs
PAS	Patient activity scale
PJI	Periprosthetic joint infections
PMR	Polymyalgia rheumatica
PPV	Positive predictive value
RA	Rheumatoid arthritis
RACGP	Rheumatoid Arthritis Working Group of The Royal Australian College of General Practitioners
RAPID3	Routine assessment of patient index data 3
RD	Rheumatic disease
RDT	Rapid diagnostic test
RF	Rheumatoid factor
SAA	Spondylitis Association of America
SDAI	Simplified disease activity index
SIRS	Systemic inflammatory response syndrome
SLE	Systemic lupus erythematosus
TSH	Thyroid-stimulating hormone
VASDA	Visual analog scale disease activity
VASQOL	VAS quality of life
WHO	World Health Organization

Scientific Background

Conditions Associated with Acute Inflammatory Responses

Diseases most associated with an acute inflammatory response measured by C-reactive protein (CRP) and/or erythrocyte sedimentation rate (ESR) include arthritis, especially rheumatoid arthritis (RA), polymyalgia rheumatica (PMR), giant cell arteritis (GCA), systemic lupus erythematosus (SLE), cardiovascular disease (CVD) (Kushner, 2024), and Hodgkin lymphoma (HL) (NCCN, 2024b). RA is a systemic polyarthritis that can lead to joint loss as well as tendon and ligament deformation to the point of affecting day-to-day living. The diagnosis of RA can be made in a patient "with inflammatory arthritis involving three or more joints, positive RF [rheumatoid factor] and/or anti-citrullinated peptide/protein antibody, disease duration of more than six weeks, and elevated CRP or ESR, but without evidence of diseases with similar clinical features" (Baker, 2024). PMR "is an inflammatory rheumatic condition characterized clinically by aching and morning stiffness about the shoulders, hip girdle, and neck" (Salvarani & Muratore, 2023a). PMR is frequently associated with GCA (also known as Horton disease), which is vasculitis of medium-to-large blood vessels and can include the aorta and cranial arteries. Cranial arteritis can lead to permanent vision loss. An estimated 40-50% of patients with GCA also suffer from PMR whereas 15% of all PMR patients are also diagnosed with GCA. Due to inflammation of the aorta and aortic branches, aortic aneurysm and aortic dissection can occur in patients with GCA (Salvarani & Muratore, 2023b). In both PMR and GCA, ESR and CRP levels are typically elevated. SLE "is a complex autoimmune disease with chronic relapsing-remitting course and variable manifestations leading a spectrum from mild mucocutaneous to devastating, life-threatening illness… Epigenetic modifications mediate the effect of the environment on immunologic responses, eventually leading to

an inflammatory, autoimmune, multi-systemic disease characterized by autoantibody production and tissue injury" (Gergianaki & Bertsias, 2018). Since patients with SLE can be prone to infection, ESR and CRP may be used in monitoring inflammation (Kushner, 2024). CVD is a very common inflammatory disorder in the United States. Although serum CRP is a non-specific inflammatory marker and is not a causative agent of CVD, serum CRP can be used as a biomarker for CVD (Black et al., 2004; Kushner, 2024). Hodgkin lymphoma accounts for 10% of lymphomas and is characterized as a B-cell lymphoma "containing a minority of neoplastic cells (Reed-Sternberg cells and their variants) in an inflammatory background" (Aster & Pozdnyakova, 2022). ESR is elevated in HL, and an ESR ≥50 is considered as an "early-stage unfavorable factor" (NCCN, 2024b).

Erythrocyte Sedimentation Rate (ESR)

Erythrocyte sedimentation rate (ESR) is a common laboratory method used to monitor general inflammation. ESR is used to analyze many different conditions, including RA, SLE, arteritis, PMR (Kushner, 2024; Wu et al., 2010). The simple Westergren method of ESR consists of measuring the distance a blood sample travels in a tube within one hour. The International Council for Standardization in Hematology (ICSH) established a calibration reference to this method using citrate-diluted samples. Automated ESR methods have been established; however, some of these analyzers use different dilution solutions, such as EDTA, rather than citrate. EDTA is commonly used as an anticoagulant in hematology measurements whereas the use of citrate is less prevalent. Horsti et al. (2010) compared blood samples from 200 patients using the traditional Westergren method versus an EDTA-based method. Their data has an R^2 value of only 0.72 and 55 subjects had a difference of over 30%, clearly indicating that ESR is significantly affected by sample preparation methods (Horsti et al., 2010). ESR can also be affected by red blood cell morphology, ambient conditions (such as high room temperature or tilting of the ESR tube), anemia, renal disease, obesity, heart failure, and hypofibrinogenemia (Kushner, 2024; Taylor & Deleuran, 2024).

More, ESR may be affected by noninflammatory factors, thus reducing its specificity for inflammatory processes. Noninflammatory biological factors and environmental conditions can increase a sample's observed ESR. If the serum sample contains elevated concentrations of ions or charged proteins, an elevated ESR may occur; for example, an increase in positively charged plasma proteins could result in agglutination of erythrocytes within a sample for rapid sedimentation (Hale et al., 2019).

The ICSH established a Working Group to investigate the ESR methodology used in laboratories; the findings of this working group were published in 2017. Data from over 6000 laboratories on four different continents was examined. Of the laboratories included in the study, only 28% used the "gold standard" Westergren method exclusively (i.e. the method with the established validation by the ICSH) "while 72% of sites used modified or alternate methods." The data obtained from the new methodologies could deviate from the Westergren method by up to 142% and could differ "from each other of up to 42%." The ICSH released recommendations based up the results of these studies. One such recommendation for labs using the non-Westergren method of ESR is to "consider adding an interpretative comment to every result stating that 'This result was obtained with an ESR instrument that is not based on the standard Westergren method. The sensitivity and specificity of this method for various disease states may be different from the standard Westergren method'" (Kratz et al., 2017).

Besides the Westergren method, other methods have been developed to measure ESR including the Zeta sedimentation ratio, Wintrobe's method, and micro-ESR. In a validation study, Shaikh discussed the use of the Ves-Matic Cube 30 analyzer to address the drawbacks of the Westergren method such as contamination risk, the significant blood volume required, and increased duration of analysis. A strong

positive correlation was observed between Westergren and Ves-Matic methods with Spearman's coefficient of 0.97. The study concluded that Ves-Matic Cube 30 analyzer can be used in high workload clinical settings for ESR measurement as the generated results were in concordance with those from the Westergren method.

C-reactive Protein (CRP)

C-reactive protein (CRP) was first discovered in the early twentieth century when it was isolated in a co-precipitation reaction with the pneumococcal C polysaccharide. The polysaccharide component bound by CRP later was identified to be phosphocholine. Since then, studies have shown that CRP can bind ligands other than bacterial cell wall components. During an acute inflammatory response, hepatocytes can upregulate CRP synthesis more than 1000-fold. The increase in serum CRP "after tissue injury or infection suggests that it contributes to host defense and that it is part of the innate immune response" (Black et al., 2004). Determining CRP concentration and fluctuations in plasma CRP can be useful in monitoring inflammatory response; however, what dictates "normal" CRP levels is of debate since CRP concentrations can vary considerably between individuals, people groups, and laboratory testing methodology. The units used to denote CRP concentrations also vary between laboratories (Kushner, 2024).

Clinical Validity and Utility of CRP and ESR in Measuring Inflammatory Processes

Both CRP and ESR have been used to monitor RA. Elevated CRP and ESR does correlate to observed radiologic damage in RA. Unlike ESR, CRP can be evaluated in stored serum. This could be advantageous due to the time constraints of ESR testing (Taylor & Deleuran, 2024). A 2009 study by Crowson et al. (2009) show that the use of both ESR and CRP testing in the case of RA is not warranted. Data from three randomized trials of 1247 RA patients was examined. "Where available, the CRP alone may be preferred for disease activity assessment as a simple, validated, reproducible, non age-dependent test" (Crowson et al., 2009). Since both ESR and CRP have been incorporated into composite scoring for RA, the elimination of one or the other will not hinder the quantitative evaluation of the patient using a composite scoring system such as DAS (Disease Activity Score) or SDAI (Simplified Disease Activity Index). A 2015 Danish study clearly shows that the data obtained in DAS using either ESR or CRP "are interchangeable when assessing RA patients and the two versions of DAS28 are comparable" (Nielung et al., 2015). This study compared the baseline data and one-year follow-up of 109 different patients with RA using the DAS28-ESR and DAS28-CRP. Using the EULAR (European League Against Rheumatism) response criteria, only 14 patients show a divergence between using the ESR and CRP methods. Of those 14, "12 showed a better response (in terms of responder category) using DAS28-CRP, while two patients showed a better response using DAS28-ESR." However, a 2006 study by Fransen and van Riel (2006) show that it is still possible for a patient to have a high number of swollen joints and yet receive a low DAS28-ESR score within the remission range due to a low ESR value since ESR has a significant weight on the DAS28-ESR algorithm (Fransen & van Riel, 2006). This study did not include CRP measurements to compare its validity to that of the DAS28-ESR. Another study released in 2010 (Hensor et al., 2010) shows that the DAS28-CRP could also underestimate RA remission rates since those values are usually lower than the corresponding DAS28-ESR values, but the discrepancy is not significant if age and gender are added as factors into the DAS28-CRP methodology. To confound issues, "newer biologic agents that target specific inflammatory cytokines are differentially reflected in the ESR and CRP and may therefore disproportionately deflate the composite score" (Anderson et al., 2012).

Erythrocyte sedimentation rate cannot be used to predict RA as a screening method. Suarez-Almazor and colleagues investigated the predictive value of ESR for connective tissue diseases (CTD) and RA.

Their review of 711 records by more than 300 different primary care physicians in Alberta show that ESR positively predicted 35% for CTD and only 17% for cases of RA. For SLE, the positive predictive value for ESR was even lower at only three percent. CRP testing was not included in this study. The authors note that "most tests were negative, and were often requested in patients without CTD, resulting in low positive predictive values and questionable clinical utility" (Suarez-Almazor et al., 1998). A study by Keenan et al. (2008) compared the utilization of ESR and CRP in RA, SLE, and osteoarthritis. The data showed that for the 188 patients with RA, the number of patients with both ESR and CRP elevated were statistically the same as those with normal test levels or those with only one test elevated. Conclusions stated "that another look at the role of ESR and CRP as markers of inflammation in RA patients seen in routine care may be in order" (Keenan et al., 2008).

Bitik et al. (2015) researched the use of elevated ESR and CRP levels in distinguishing the definitive diagnosis of a rheumatic disorder from patients with nonspecific inflammation. In their study of 112 patients, 47 had a previously diagnosed rheumatic disorder and 65 had no history of a rheumatism. Of the 65 patients with no history of a rheumatic disorder, 52.3% were diagnosed with a new rheumatic disorder with PMR/GCA comprising 38.2%, while 47.7% had a non-rheumatic diagnosis. Within this latter group, only the "CRP levels were significantly higher in infections when compared with new onset RD (rheumatic disease) or malignancies ($p < 0.05$)" (Bitik et al., 2015). The ESR levels between the three groups were statistically insignificant. This indicates that CRP is more sensitive to acute infections than ESR. The authors state that "although ESR and CRP levels have a very low specificity in differentiating between these conditions, in cases of unusually high levels of CRP (especially above 200), more consideration should be given to infections or malignancies."

A 2014 study of 60 different PMR patients compared the efficacy of ESR and CRP in assessing disease activity versus patient-reported outcomes and plasma fibrinogen. In this study, the VASDA (Visual analog scale disease activity) and VASQOL (VAS quality of life), two patient-reported outcome methods, were the most responsive to changes in disease activity. Of the serum biomarkers, fibrinogen, ESR, and CRP, fibrinogen was the most accurate with a correlation coefficient of 1.63 whereas 1.2 and 1.05 were the correlation coefficients of ESR and CRP, respectively. These data suggest that plasma fibrinogen would be a more sensitive measure of PMR disease activity as compared to either ESR or CRP (McCarthy et al., 2014).

A two-year retrospective study released in 2010 (Ernst et al., 2010) researched the validity of using either ESR and/or CRP in assessing septic joints. This study consisted of 163 patients and included both genders as well as patients with alcohol or drug histories. The mean ESR value for the 119 control non-septic joints was 46 while the septic joint mean ESR value was 57, which was however, the mean CRP value was 13 in the septic joints and 8.5 in the non-septic joints. The conclusion of the authors is that "CRP is helpful in determining the presence of a septic joint; ESR is not" (Ernst et al., 2010).

Erythrocyte sedimentation rate is used in determining the algorithm to follow in the treatment of Hodgkin lymphoma (CHL). For example, in stage 1A CHL, a patient with an ESR <50 would follow either the NCCN HODG-3 or HODG-4 algorithm with an initial 2-3 cycles of ABVD (Adriamycin, bleomycin, vinblastine, dacarbazine) most likely whereas a stage 1A patient with an ESR ≥50 would follow the NCCN HODG-6 algorithm with a possible involved-site radiation therapy (ISRT) initially along with the chemotherapy since an ESR ≥50 is considered an "unfavorable factor" (NCCN, 2024b).

C-reactive Protein elevation is associated with a number of inflammatory disorders (including RA), tissue damage (such as after a myocardial infarction), as well as bacterial infections; however, CRP levels in SLE do not mirror disease progression (Kushner, 2024). Even during cases of severe disease phenotypes, CRP

levels can be normal to modestly increased. One possible reason is CRP suppression by type I interferons, which are increased in SLE. Another possibility is that low concentrations of wildtype CRP play a role in lupus. "Three lines of investigation have raised the possibility that low plasma levels of CRP may be related to the pathogenesis of SLE: 1) an association between SLE and several CRP genetic polymorphisms, at least one of which is associated with low CRP levels, 2) the possibility that low CRP levels may contribute to defective clearance of autoantigens during apoptosis, and 3) the therapeutic efficacy of CRP in mouse models of SLE" (Gaitonde et al., 2008). Also, CRP and anti-CRP may form large complexes in patients with SLE, which could also decrease the serum concentrations of free CRP (Gordon et al., 2018). A study by O'Neill and colleagues in 2010 show that anti-CRP levels are directly proportional in an increase to disease activity (32.6, 24.8, and 16.8 AU, respectively, for high activity, low activity, and control groups) and that anti-CRP levels were above the upper limit of normal in 26.3% of the high activity cases versus only 12.8% for the low activity cases (O'Neill et al., 2010). Patients with SLE usually have elevated ESR, but this elevation may be due to persistent polyclonal hypergammaglobulinemia (increased production of several different immunoglobulins) (Gordon et al., 2018).

Periprosthetic joint infections (PJI) may also benefit from testing of CRP and ESR. Joint arthroplasties (replacements) are typically performed in response to joint damage or destruction and commonly involve areas such as the hip, knee, or shoulder. Up to 2% of total knee replacements may become infected. Common signs of infection are present in PJI such as joint pain or warmth at the incision site, and microbiological cultures may be performed to confirm the diagnosis. CRP and ESR have been suggested as supportive biomarkers in cases where a definitive diagnosis cannot be made. CRP and ESR are considered minor clinical diagnostic criteria in some definitions of PJI, but due to the ubiquity of these markers, their levels are usually interpreted cautiously (Baddour & Chen, 2023).

Berbari et al. (2010) performed a meta-analysis of inflammatory markers in prosthetic joint infection. A total of 30 studies including 3909 revision total hip or knee replacements were assessed, and of the 3909 operations, 1270 infections occurred. CRP was included in 23 of 30 studies, and its diagnostic odds ratio was found to be 13.1. ESR was included in 25 of 30 studies, and its diagnostic odds ratio was calculated to be 7.2. Interleukin-6 was found to be the best marker of all markers addressed, albeit with only three studies (Berbari et al., 2010).

Perez-Prieto et al. (2017) examined the performance of CRP and ESR for PJI diagnosis. A total of 73 patients were included in the study. Preoperative CRP levels were found to be normal in 23 patients, and of those 23 patients, 17 patients also had normal ESR levels. Further, 16 patients with normal CRP levels were found to have "low-virulence" organisms (such as *Propionibacterium acnes* and coagulase-negative staphylococci) present. Overall, the authors found that 23% of the patients included in this study would not have been diagnosed with PJI according to the American Association of Orthopaedic Surgeons (AAOS) guidelines or the Musculoskeletal Infection Society definition (Perez-Prieto et al., 2017).

Kheir et al. (2018) evaluated the accuracy of inflammatory markers in diagnosis periprosthetic joint infections (PJI). A total of "549 periprosthetic joint infection cases and 653 aseptic total joint arthroplasty revisions" were reviewed. The sensitivity of ESR to diagnose PJI was 0.85 and 0.88 for CRP. ESR was also elevated in antibiotic-resistant strains of bacteria compared to culture-negative cases. For CRP, gram-negative species had higher levels of CRP than culture-negative cases. Overall, the authors concluded that both ESR and CRP had higher false-negative levels than previously reported (Kheir et al., 2018).

Hamann et al. (2019) compared the DAS28-ESR and DAS28-CRP to determine the impact on disease activity stratification in RA. A total of 31,074 paired data sets were included in this study and were

obtained from the British Society for Rheumatology Biologics Register for RA. Results showed that "DAS28-CRP scores were ~0.3 lower than DAS28-ESR overall, with greatest differences for women (-0.35) and patients over 50 years old (-0.34). Mean male DAS28-CRP scores were 0.15 less than corresponding DAS28-ESR scores" (Hamann et al., 2019). When DAS28-CRP data is adjusted by gender, significant agreement ($P<0.001$) is seen with DAS28-CRP and DAS28-ESR scores.

Bingham et al. (2019) measured the specificity and sensitivity of ESR and CRP when screening for a PJI infection using the standard MSIS cutoff of 30 mm/h and 10 mg/L, respectively. The researchers also hoped to determine the optimal CRP and ESR cutoff to achieve a ≥95% sensitivity. Data from a total of 81 PJI patients and 83 noninfected arthroplasty patients was analyzed for this study. Results showed that "The ESR cutoff that resulted in a sensitivity ≥ to 95% (95% CI: 85.2-97.6%) was 10 mm/h, and the CRP cutoff that resulted in a sensitivity ≥ to 95% (95% CI: 87.1-98.4%) was 5 mg/L. The sensitivity and specificity with a combined ESR and CRP of 10 mm/h and 5 mg/L was 100%" (Bingham et al., 2019). The authors note that the accepted cutoff of 30 mm/h and 10 mg/L leads to a high number of false positives and low sensitivity; these thresholds therefore need to be reevaluated.

In a prospective cohort study, Watson et al. (2019) compared the diagnostic value of CRP and ESR and evaluated whether measuring two inflammatory markers increases accuracy. For each test, sensitivity, specificity, PPV, NPV, and AUC were calculated. A total of 136,961 patients with inflammatory testing were measured of which 61.2% had a single marker measured and 38.8% had multiple markers measured. CRP and ESR were broadly similar in terms of sensitivity, specificity, PPV, and NPV. However, CRP had the highest overall AUC of 0.682 while the AUC for ESR was 0.589. Adding a second test did little improvement in AUC. When CRP and ESR were both tested for, the AUC increased from 0.682 to 0.688. Overall, the authors conclude that "Testing multiple inflammatory markers simultaneously does not increase ability to rule out disease and should generally be avoided. CRP has marginally superior diagnostic accuracy for infections, and is equivalent for autoimmune conditions and cancers, so should generally be the first-line test" (Watson et al., 2019).

In a cross-sectional study, Sherkatolabbasieh et al. (2020) investigated platelet count, ESR, and CRP levels in pediatric patients with inflammatory disease. A total of 150 children (75 male and 75 female) with diagnosis of infectious and inflammatory diseases were included in the study. Platelet count, ESR, and CRP levels were measured at the time of hospitalization and at discharge. At time of hospitalization, all 150 children had abnormal ESR levels, 73.3% had abnormal CRP levels, and 8% had abnormal platelet levels. At time of discharge, only one patient recovered to normal ESR levels, 88% had normal CRP, and 93.3% had normal platelet count. The Fisher exact test showed a significant relationship between platelet count and CRP levels at the time of discharge ($p < 0.0002$) and admission ($p < 0.007$), especially in the female patients. CRP levels were significantly higher in the female patients and changes in platelet count were more prevalent. No relation between platelet count and ESR was observed at admission and discharge. This study found that there are differences in inflammatory markers between the two sexes. The authors conclude that this study showed significant correlation between CRP and platelet levels in girls and CRP level measurement is useful in treatment follow up (Sherkatolabbasieh et al., 2020).

Alende-Castro et al. (2021) studied the use of CRP vs ESR in 1472 patients with no inflammatory conditions. All participants were measured for ESR, CRP and IL-6 concentrations. A total of 74.9% of participants showed normal CRP and ESR values, 4.6% showed high ESR and CRP values, and 13.8% showed high CRP but normal ESR values. Participants with high ESR/CRP values also were of older age, and reported high alcohol consumption, low physical activity, high BMI, and the presence of metabolic syndrome. In those patients who had high CRP but normal ESR, BMI seemed to be the main determinant of CRP concentrations. The authors concluded that "In this general adult population with no overt

inflammatory disease, the discordant pattern of high ESR and normal CRP was associated with greater age, whereas the pattern of high CRP and normal ESR was associated with higher BMI" (Alende-Castro et al., 2021).

In a retrospective study, Christopher studied the use of ESR/CRP ratio to differentiate acute vs chronic periprosthetic joint infections. A total of 147 patients (81 acute and 66 chronic) were measured for ESR and CRP concentrations. The mean ESR/CRP ratio in acute patients was 0.48 compared to 2.87 in chronic patients. The ideal cutoff value was 0.96 for ESR/CRP to predict a chronic (>0.96) vs. acute (<0.96) PJI. The sensitivity at this value was 0.74 and the specificity was 0.90. The authors conclude that "The ESR/CRP ratio may help determine the duration of PJI in uncertain cases. This metric may give arthroplasty surgeons more confidence in defining the duration of the PJI and therefore aid in treatment selection" (Christopher et al., 2021).

Dhudasia et al. (2022) conducted a retrospective cohort study to determine the clinical utility of CRP in diagnosing early-onset sepsis and assessing patient outcomes. The patient sample included over 10,000 infants admitted to the neonatal intensive care units from 2009-2014, when CRP was used routinely. The cutoff utilized as ≥10mg/L for diagnosis of "culture-confirmed early-onset sepsis." Based on when the CRP was obtained from the blood culture, which was taken at three days of birth, the results yielded varying specificities and sensitivities. If the CRP was obtained at ± four hours, the sensitivity was 41.7%, specificity 89.9%, and positive likelihood ratio was 4.12. When obtained 24-72 hours later, the sensitivity became 89.5%, but specificity decreased to 55.7% and positive likelihood ratio to 2.02. During this time of routine CRP testing, there were higher rates of early-onset sepsis evaluation, antibiotic initiation, and antibiotic prolongation "in the absence of early-onset sepsis," but no difference in time to detection and in-hospital mortality with a period of non-routine CRP testing. The researchers ultimately concluded that the diagnostic performance of CRP in diagnosing early-onset sepsis was insufficient to warrant routine testing, as patient outcomes were not significantly affected with the elimination of routine CRP testing. Other factors with higher sensitivities, specificities, and positive likelihood ratios need to be included in the evaluation (Dhudasia et al., 2022).

While the search for the optimal biomarker in inflammatory bowel disease (IBD) continues, the most widely available biomarkers in current clinical practice include serum testing with CRP and ESR (Clough et al., 2024; Muresan & Slevin, 2024). CRP is frequently used by primary care clinicians to differentiate between IBD and irritable bowel syndrome (IBS). According to Clough et al. (2024), "CRP is limited as a biomarker in IBD by its lack of specificity, with its expression upregulated in numerous infective and inflammatory pathologies, thus limiting its usefulness in distinguishing between IBD and other differential diagnoses. Its utility in IBD is largely as an adjunct to clinical and endoscopic findings." However, exclusion of IBD should not be made based solely on a normal CRP but in combination with clinical assessment and other markers with better sensitivity (Clough et al., 2024).

Guidelines and Recommendations

World Health Organization (WHO)

On May 16, 2018, the WHO released their first edition of the *Model List of Essential In Vitro Diagnostics* (EDL) "to advance universal health coverage, address health emergencies, and promote healthier populations." This list of in vitro diagnostics (IVD) is to be used as a reference of the essential diagnostic tools for laboratories to complement their Model List of Essential Medicines. With respect to the diagnostic tool "to detect inflammation as an indicator of various conditions," the WHO recommends

CRP either in an EIA (enzyme immunoassay) or RDT (rapid diagnostic test) assay format. The specimen type can be venous whole blood, serum, or plasma.

In 2019, the WHO released the *Second WHO Model List of Essential In Vitro Diagnostics.* In a table titled *General IVDs for Use in Clinical Laboratories*, CRP is once again listed. The WHO now recommends CRP in an RDT, latex agglutination assay or immunoassay format (WHO, 2019).

In 2020, the WHO released *the selection and use of essential in vitro diagnostics*, which included the third WHO model list. In the section on "General IVDs for community settings and health facilities without laboratories," the WHO performed an evaluation of utilizing ESR "to aid diagnosis and monitoring of certain infections and immune diseases; and as an alternative to a C-reactive protein (CRP) test where this is not available." In their table, they recommend using the Westergren assay format with sampling from venous whole blood. The WHO ultimately concluded that despite several guidelines recommending ESR to aid in diagnosing several inflammatory diseases, "there is no strong evidence supporting ESR as an essential test" since there are also high rates of false positives and false negatives. They conclude that CRP "should remain the preferred choice of test," except in cases of systemic lupus erythematosus and low-grade bone and joint infections since "there is evidence that the condition elevates ESR without causing a raise in CRP." As of this meeting, CRP now has the purpose "to monitor response to treatment" in addition to "detect inflammation as an indicator of various response conditions," and can be assayed as RDT, latex agglutination assay, and immunoassay with sampling venous whole blood, serum, and plasma (WHO, 2020).

National Comprehensive Cancer Network (NCCN)

The NCCN guidelines concerning Hodgkin Lymphoma uses ESR as a diagnostic tool in characterizing the stage/risk classification of Classic Hodgkin Lymphoma (CHL) as well as the primary treatment of the disease. In the diagnosis/workup of Hodgkin Lymphoma in adults (age ≥18 years) (recommendation 2A), they list erythrocyte sedimentation rate (ESR) as "essential" and that ESR should be tested within six months of diagnosis.

In the guidelines concerning follow-up after completion of treatment up to five years, the NCCN (2024b) recommends obtaining an interim history and physical "every 3-6 [months] for 1-2 [years], then every 6-12 [months] until year 3, then annually," as well as laboratory studies, which included a "[complete blood count], platelets, chemistry profile, and ESR if elevated at time of initial diagnosis" with the same timeline. ESR is also used in determining the dosage of involved-site radiation therapy (ISRT). "A dose of 20 Gy following ABVD X 2 is sufficient if the patient has non-bulky stage I-IIA disease with an ESR <50, no extralymphatic lesions, and only one or two lymph node regions involved." An ESR ≥50 is considered as an "unfavorable risk factor" for stages I and II Hodgkin Lymphoma along with B symptoms. Please note that the NCCN guidelines concerning Hodgkin Lymphoma do not contain any information concerning the use of CRP as a diagnostic or prognostic tool (NCCN, 2024b).

In the NCCN guidelines concerning Castleman Disease, the NCCN recommend regarding diagnostic criteria for idiopathic MCD (Multicentric Castleman Disease), minor diagnostic criteria include elevated CRP (>10 mg/L) or ESR (>15 mm/h) where an "Evaluation of CRP is mandatory and tracking CRP levels is highly recommended, but ESR will be accepted if CRP is not available" (NCCN, 2024a).

In the NCCN guidelines concerning the T-cell lymphomas, they state that the "evaluation of serologic markers such as rheumatoid factor (RF), antinuclear antibodies (ANA), and erythrocyte sedimentation rate (ESR) is useful in patients with autoimmune disease" (NCCN, 2024c). The guidelines concerning T-

cell lymphomas do not mention the diagnostic or prognostic use of CRP.

American Society for Clinical Pathology (ASCP)

In the Choosing Wisely site of the ABIM Foundation, the ASCP released the recommendation to not "order an erythrocyte sedimentation rate (ESR) to look for inflammation in patients with undiagnosed conditions. Order a C-reactive protein (CRP) to detect acute phase inflammation" due to the higher sensitivity and specificity of CRP for acute phase of inflammation. "In the first 24 hours of a disease process, the CRP will be elevated, while the ESR may be normal. If the source of inflammation is removed, the CRP will return to normal within a day or so, while the ESR will remain elevated for several days until excess fibrinogen is removed from the serum" (ASCP, 2020).

European League Against Rheumatism (EULAR)

In 2009, EULAR issued their recommendations concerning the management of large vessel vasculitis, which is comprised of two distinct conditions: giant cell arteritis and Takayasu arteritis. With a "Level of Evidence 3, Strength of recommendation C", they recommend "monitoring of therapy for large vessel vasculitis should be clinical and supported by measurement of inflammatory markers…. For patients with giant cell arteritis, a relapse is usually associated with a rise in ESR and CRP" (Mukhtyar et al., 2009). In this paper, no mention of the frequency of ESR and/or CRP testing is mentioned.

In the 2018 update of the EULAR recommendations for the management of large vessel vasculitis, the guidelines state that regular follow-up and monitoring of disease activity is recommended in patients with large vessel vasculitis, primarily based on symptoms, clinical findings and ESR/CRP levels. "Visits should include clinical monitoring and measurement of ESR and CRP." These routine follow-up visits could be scheduled every 1–3 months during the first year and in 3–6 months intervals afterwards (Hellmich et al., 2020).

In 2013 in *EULAR recommendations for the use of imaging of the joints in the clinical management of rheumatoid arthritis* (Colebatch et al., 2013), they state that "baseline inflammatory disease measured by scintigraphy appears to be associated with radiographic progression. In addition, multiple regression analysis has demonstrated that progression of radiographic joint destruction was primarily predicted by ^{99m}Tc-IgG scintigraphy; joint swelling, ESR and IgM RF (Rheumatoid Factor) were not predictive. This suggests that scintigraphy may be superior to conventional clinical and laboratory measurements in the prediction of joint destruction." This set of guidelines did not include any mention concerning CRP or the frequency of ESR testing.

In 2015, EULAR and the American College of Rheumatology (ACR) issued joint recommendations concerning the management of polymyalgia rheumatica (PMR) (Dejaco et al., 2015). Within their recommendations, they list assessments that "every case of PMR should have…prior to the prescription of therapy (primary or secondary care)." They include a basic laboratory workup "to exclude mimicking conditions and establish a baseline for monitoring of therapy", and they state that this includes "rheumatoid factor and/or anti-cyclic citrullinated peptide antibodies (ACPA), C-reactive protein and/or erythrocyte sedimentation rate (ESR), blood count, glucose, creatinine, liver function tests, bone profile (including calcium, alkaline phosphatase) and dipstick urinalysis." They do not state a specific preference of either CRP or ESR nor do they state the frequency of testing.

EULAR in 2016 updated their 2007 recommendations concerning the management of early arthritis (Combe et al., 2017). The 2016 updates included the following recommendation: "Monitoring of disease activity should include tender and swollen joint counts, patient and physician global assessments, ESR

and CRP, usually by applying a composite measure. Arthritis activity should be assessed at 1-month to 3-month intervals until the treatment target has been reached." The recommendation concerning including both ESR and CRP did not change between the 2016 and 2007 recommendations. Within the discussion of the recommendations, they state, "In every patient with active arthritis, closely monitoring disease activity is now considered of particular importance in the therapeutic strategy to provide a good outcome. . . Monitoring disease activity should be as frequent as the level of disease activity mandates, usually every 1-3 months, then potentially less frequently (such as every 6-12 months) once the treatment target has been achieved. Nevertheless, three changes were proposed to this item…. First, a composite measure was recommended as the method of choice to monitor disease activity; second, a specific time frame for monitoring structural damage was deliberately left out and third, patient-reported outcomes were expanded beyond functional assessments" (Combe et al., 2017).

In 2018, EULAR issued *EULAR recommendations for the use of imaging in large vessel vasculitis in clinical practice* (Dejaco et al., 2018). They make no recommendation concerning the preference of ESR or CRP nor do they state the frequency of testing; they do state "in patients with a high clinical suspicion of GCA (>50%), for example, in case of new-onset headache, visual symptoms, jaw claudication and elevated erythrocyte sedimentation rate (ESR) and C reactive protein, a positive ultrasound would result in a post-test probability of >95%."

American College of Rheumatology (ACR)

In 2012, ACR released their recommendations concerning the clinical practice of using disease activity measures of rheumatoid arthritis (RA) (Anderson et al., 2012). The recommend using the Disease Activity Score with 28-joint counts (DAS28), the Clinical Disease Activity Index, the Patient Activity Scale (PAS), the PAS-II, the Simplified Disease Activity Index (SDAI), and Routine Assessment of Patient Index Data with three measures. The DAS28 is a composite test that can use either CRP or ESR data. The ACR states that both the CRP or ESR used in the DAS28 have been validated in RA. Of the six activity measures recommended by the ACR, only DAS28 received "excellent" recommendations for all three psychometric properties—reliability, validity, and responsiveness. Within the guidelines, the ACR also issued the scores corresponding to remission, low/minimal, moderate, and high/severe RA for all the disease activity measures, including the DAS28, as well as the mathematical formula using either CRP or ESR data to determine the DAS28. CRP is also used in the SDAI; however, the SDAI is rated as "good" for reliability because they state that "test-retest reliability for composite has not been evaluated" for the SDAI. No mention of frequency of testing is made. They do note that the "inclusion of acute-phase reactants in the DAS28 and SDAI complicates the logistics and timing using these measures in point-of-care clinical decision making. Although these measures have traditionally been used in clinical trials, academic medical centers, and large multispecialty clinics, logistical barriers have likely delayed their widespread adoption in smaller practice settings" (Anderson et al., 2012).

The ACR in 2015 (Singh et al., 2015) issued guidelines for the treatment of RA. While not specifying a preference of either CRP or ESR in diagnosing or predicting the prognosis of RA, they do state in their "Key provisos and principles" that "functional status assessment using a standardized, validated measure should be performed routinely for RA patients, at least once per year, but more frequently if disease is active." They also state that disease activity be measured using ACR-validated scales, including the DAS28 and/or SDAI. Moreover, they define RA remission as "a tender joint count, swollen joint count, C-reactive protein level (mg/dl), and patient global assessment of ≤1 each or a Simplified DAS of ≤3.3, 1 of 6 ACR-endorsed disease activity measures".

Also, in 2015 (but published in 2016), the ACR and the Spondylitis Association of America (SAA) issued their joint recommendations concerning the treatment of ankylosing spondylitis (AS) and nonradiographic axial spondyloarthritis (Ward et al., 2016). Regarding "the treatment of patients with either active or stable AS…we conditionally recommend regular-interval use and monitoring of the CRP concentrations or erythrocyte sedimentation rate (ESR) over usual care without regular CRP or ESR monitoring." This received a "very low-quality evidence; vote 100% agreement" rating. They do make note that as of the time of publication "no studies addressed the effect of routine monitoring of a disease activity measure" but that "the panel thought that monitoring would be most helpful in patients with active symptoms as a guide to treatment." Testing is not required for every clinic visit. The two organizations reaffirm the same recommendations in their 2019 update (Ward et al., 2019).

In 2019, updated recommendations by the RA disease activity measures working group were published by England et al. (2019). Recommended tests include the Clinical Disease Activity Index (CDAI), the Simplified Disease Activity Index (SDAI), the Routine Assessment of Patient Index Data 3 (RAPID3), and the 28-Joint Disease Activity Score (DAS28). As noted above, the DAS28 is a composite test that can use either CRP or ESR data. The ACR states that both the CRP or ESR used in the DAS28 have been validated in RA. Updates to the management of rheumatoid arthritis were released by the ACR in 2022, but no mention of CRP or ESR were made (Arnold, 2022).

In 2021, the ACR published a guideline to provide evidence-based recommendations and expert guidance for the management of giant cell arteritis (GCA). They present 22 recommendations and two ungraded position statements for GCA and note that all but one of the recommendations are conditional due to very low- to low-quality evidence. They break these recommendations down into categories, including diagnostic testing, medical management, surgical intervention, and clinical/laboratory monitoring. All diagnostic recommendations involve biopsy or imaging- they do not recommend the use of CRP or ESR for diagnosis of GCA. However, they do recommend inflammation marker monitoring as part of clinical/laboratory monitoring. They define clinical monitoring as "assessing for clinical signs and symptoms of active disease, obtaining 4 extremity blood pressures, and obtaining clinical laboratory results, including inflammation marker levels", with inflammation markers further defined as being CRP and ESR:

"Recommendation: For patients with GCA in apparent clinical remission, we strongly recommend long-term clinical monitoring over no clinical monitoring: The optimal frequency and length of monitoring are not well established and depend on factors including the duration of remission, site of involvement, risk of disease progression, whether the patient is receiving immunosuppressive therapy, and reliability of the patient to report new signs or symptoms. Clinical monitoring may include history taking, examinations, and laboratory and imaging studies. This is a strong recommendation given the minimal risks and potential catastrophic outcomes if monitoring is not performed.

Recommendation: For patients with GCA who have an increase in levels of inflammation markers alone, we conditionally recommend clinical observation and monitoring without escalation of immunosuppressive therapy. Increases in levels of inflammation markers such as erythrocyte sedimentation rate and C-reactive protein can be nonspecific (69). Therefore, increasing immunosuppressive therapy is not warranted in the setting of increased levels of inflammation markers in the absence of other signs of disease activity. However, these increased levels may warrant more frequent clinical and/or radiographic assessments for active disease" (Maz et al., 2021)

American Academy of Family Physicians (AAFP)

In 2013, the AAFP released *Recognition and Management of Polymyalgia Rheumatica and Giant Cell Arteritis*. For polymyalgia rheumatica (PMR), they note that "a normal ESR is found in 6% to 20% of persons with [PMR], although in those cases C-reactive protein level is elevated. ESR predicts relapse more reliably, but C-reactive protein is more sensitive, and is less affected by age and other factors." For giant cell arteritis (GCA), ESR is elevated in up to 89% of patients, but the sensitivity and specificity increase to 99% and 97%, respectively, if both ESR and CRP are tested. Regardless of using either ESR or CRP testing, the AAFP recommends that either ESR or CRP is tested at each clinic visit for patients with either PMR or GCA (Caylor & Perkins, 2013).

American College of Radiology (ACR)

The ACR released their updated guidelines concerning the follow-up of Hodgkin lymphoma in 2014. They state that "limited data are available on the role of routine blood work in detecting relapses." ESR is listed as one of the tests conducted as routine blood work in follow-up of Hodgkin lymphoma. They summarize their findings as the following: "In general a majority of recurrences can be detected initially by history and physical examination rather than by routine imaging studies or blood tests such as ESR, CBC, and chemistry" (Ha et al., 2014). Four of the five variants they reviewed had ESR tests conducted one to two times per year, and the ACR rated the use of ESR as a 3, 5, 5, and 7 in these four variants where a "3" indicates "usually not appropriate," a "5" is "may be appropriate", and a "7" falls in the "usually appropriate" category.

The ACR released guidelines concerning management of multi-system inflammatory syndrome in children and devised a two-tier algorithm for diagnosis. ACR recommends routine lab tests as tier one testing, including complete blood count with manual differential, comprehensive metabolic panel, erythrocyte sedimentation rate [ESR], CRP measurement, and testing for SARS-CoV-2 by polymerase chain reaction or serology. If tier one lab results include CRP ≥5 or ESR≥40 and one suggestive lab feature such as neutrophilia, lymphopenia, thrombocytopenia, hyponatremia, or hypoalbuminemia, the child should undergo tier two testing, which involves EKG and echocardiogram (Henderson et al., 2020; Henderson et al., 2021).

The British Society for Rheumatology (BSR) & British Health Professionals in Rheumatology (BHPR)

In 2010, BSR and BHPR issued joint guidelines concerning the management of giant cell arteritis (GCA) (Dasgupta, 2010; Dasgupta, Borg, Hassan, Alexander, et al., 2010). They recommend "early recognition and diagnosis of GCA is paramount. Particular attention should be paid to the predictive features of ischaemic neuro-ophthalmic complications." As part of this diagnostic recommendation, they specifically list laboratory tests that should be included— "full blood count, urea and electrolytes, liver function tests, CRP, ESR." They note that, although elevated ESR and CRP levels are hallmarks of GCA, "GCA can occur in the face of lower levels of inflammatory markers, if the clinical picture is typical." Another specific recommendation states, "Monitoring of therapy should be clinical and supported by the measurement of inflammatory markers (C; this is a consensus statement)" and that at each visit "full blood count, ESR/CRP, urea and electrolytes, [and] glucose" lab tests be performed.

Also, in 2010, BSR and BHPR issued joint guidelines concerning the management of polymyalgia rheumatica (PMR) (Dasgupta, Borg, Hassan, Barraclough, et al., 2010). For PMR, they recommend initial lab testing for diagnosis to include either ESR and/or CRP prior to initiating long-term steroid therapy.

Also, during such therapy, they recommend monitoring either ESR or CRP every three months. This is a portion of the recommendation (B) of "vigilant monitoring of patients for response to treatment and disease activity." In the 2024 update, the guidelines readdress that "the diagnosis of PMR is based on symptoms, signs and laboratory markers with a directed search for other conditions that can mimic PMR" these laboratory markers include acute phase reactants including C-reactive protein, erythrocyte sedimentation rate and plasma viscosity. "The evidence base for monitoring and follow-up for people with PMR is lacking. The current recommendations are consensus-based and guided by expert opinion. Some guidelines suggest that follow-up frequency could be as frequent as 1–4 weeks until disease remission, while other guidelines suggest every 1–4 months in the first year of diagnosis" (Toyoda et al., 2024).

The British Society for Rheumatology (BSR)

The BSR alone issued their guidelines for the management of systemic lupus erythematosus (SLE) in 2018 (Gordon et al., 2018). For the statement "CRP low or normal unless infection," the BSR gives an overall level of evidence of 2++ with a B grade of recommendation whereas they grade the statement "ESR correlates with active lupus" a 2+ and only a C grade of recommendation. "ESR is often raised in active SLE, but can also reflect persistent polyclonal hypergammaglobulinaemia, and is not a reliable marker of disease activity…. A significantly raised CRP is more likely to indicate infection, and patients with raised CRP will need therefore to be thoroughly screened for infection, given that infection is the commonest cause of death in lupus patients. In contrast, a raised ESR does not discriminate between active lupus and infection." They recommend that CRP is tested at initial diagnosis and then every 1-3 months during active disease states. Once stabilized, then testing frequency can be every 6-12 months. They also state that CRP testing should be conducted on mothers with SLE during pregnancy, but they do not state the frequency of the testing during pregnancy. This guideline is currently in revision.

The BSR has also published guidelines on the diagnosis and treatment of giant cell arteritis (GCA). Regarding which evaluations should be performed when starting treatment, the BSR states that "When starting glucocorticoids for suspected GCA, diagnostically relevant symptoms and signs should be documented. Blood should be taken for full blood count, CRP and ESR before or immediately after commencing high-dose glucocorticoids. If GCA is strongly suspected, the first dose of glucocorticoid can be given without waiting for laboratory results" (Mackie et al., 2020). Further, the BSR provides a list of clinical assessments which should be carried out at or near a GCA diagnosis. These lists include "Measures of activity of GCA: laboratory markers of inflammation (CRP for all patients, plus either ESR or plasma viscosity) and full blood count (platelet count may be elevated in GCA)." Finally, regarding follow-up visits, "Each follow-up visit should include at least a full history, targeted physical examination and measurement of at least a full blood count, ESR and/or CRP, plus follow-up of any abnormalities relevant to the individual patient as well as drug-specific screening for toxicity" (Mackie et al., 2020). Revision for this guideline will be considered in 2024.

Canadian Rheumatology Association (CRA)

The 2012 guidelines by the CRA titled "Canadian Rheumatology Association Recommendations for Pharmacological Management of Rheumatoid Arthritis with Traditional and Biologic Disease-modifying Antirheumatic Drugs" recommends (with Level II and Strength B) "the presence of the following poor prognostic features should be assessed at baseline and considered when making treatment decisions: RF positivity, anti-CCP positivity, functional limitation, high number of swollen and tender joints, early erosions, extraarticular features, high ESR or CRP." They also recommend (with Level I and Strength A) "RA care providers should monitor disease activity as frequently as every 1 to 3 months in patients with

active RA." The disease activity should be monitored by a validated method, such as DAS28 or SDAI. The most recent updated "living guidelines" for this statement does not include prognostic features or make recommendations for factors included in treatment decisions (Hazlewood et al., 2022).

In 2018, CRA released guidelines on assessment and monitoring of Systemic Lupus Erythematosus. Regarding diagnosis, CRA recommends that best clinical practice includes a complete history and physical examination at baseline with laboratory monitoring which could possibly include (but is not limited to) the following tests: "complete blood count (CBC), liver enzymes, creatine kinase, creatinine and estimated glomerular filtration rate (eGFR), urine routine/microscopic (urinalysis), urine protein-creatinine ratio, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), complements (C3, C4), anti-dsDNA, antinuclear antibodies, antibodies to extractable nuclear antigens, antiphospholipid antibodies (aPL), lupus anticoagulant (LAC), anticardiolipin (aCL), anti-β2-glycoprotein I (anti-β2-GPI), and lipid profile. Follow up laboratory monitoring will depend on the patient's clinical status and may include CBC, eGFR, urinalysis, urine protein-creatinine ratio, CRP, and/or ESR, C3, C4, and anti-dsDNA antibodies" (Keeling et al., 2018).

Joint Task Force on Practice Parameters (JTFPP) of the Academy of Allergy, Asthma & Immunology (AAAAI); the American College of Allergy, Asthma & Immunology (ACAAI); and the Joint Council of Allergy, Asthma & Immunology

The JTFPP within their guidelines concerning the diagnosis and management of acute and chronic urticaria state, "Targeted laboratory testing based on history or physical examination findings is appropriate, and limited laboratory testing can be obtained. Limited laboratory testing includes a CBC with differential, sedimentation rate, and/or C-reactive protein, liver enzyme, and thyroid-stimulating hormone (TSH) measurement… Targeted laboratory testing based on history and/or physical examination (eg, obtaining TSH in a patient with weight gain, heat/cold intolerance, and thyromegaly) is recommended" (Bernstein et al., 2014).

National Institute for Health and Care Excellence (NICE)

The NICE first issued the guidelines concerning irritable bowel syndrome (IBS) in 2008 with updates in 2015 and 2017. In individuals who meet the IBS diagnostic criteria, they recommend ESR and CRP along with full blood count and antibody testing for celiac disease or tissue transglutaminase to exclude other possible diagnoses. They do not state anything concerning follow-up testing of either ESR or CRP (NICE, 2017).

In 2020, NICE issues guidelines concerning management of rheumatoid arthritis (RA). In adults with active RA, they recommend measuring CRP and disease activity monthly in specialist care until remission or low disease activity is achieved (NICE, 2020).

American Gastroenterological Association

In a 2019 guideline, the AGA provides recommendations on the use of ESR and CRP in patients presenting with chronic diarrhea:

- "Recommendation 2: In patients presenting with chronic diarrhea, the AGA suggests against the use of erythrocyte sedimentation rate or C-reactive protein to screen for IBD. *Conditional recommendation: low-quality evidence*" (Smalley et al., 2019).

The AGA notes that while there are "few settings where ESR should be considered as an appropriate screening test for IBD, there are some settings where the use of CRP might be a rational option. For example, if testing for fecal lactoferrin or calprotectin are either not available or not covered by insurance, the use of CRP might be considered to be a reasonable option to screen for IBD" (Smalley et al., 2019).

American Academy of Orthopaedic Surgeons (AAOS)

The AAOS notes that "Strong evidence supports the use of [ESR and CRP] to aid in the preoperative diagnosis of prosthetic joint infection (PJI)." However, the AAOS remarks that neither biomarker is perfectly accurate for PJI diagnosis and should not be used as sole tests for diagnosis. Critically, neither marker informs clinicians of the microbiology of the PJI.

These guidelines were endorsed by IDSA, the American College of Radiology, and the Society of Nuclear Medicine and Molecular Imaging (AAOS, 2019).

Pediatric Infectious Diseases Society and the Infectious Diseases Society of America

In 2021, a guideline was released on the diagnosis and management of Acute Hematogenous Osteomyelitis (AHO) in pediatrics. In children with suspected AHO, they recommend performing a serum C-reactive protein (CRP) on initial evaluation. "Serum CRP has a low accuracy to establish the diagnosis of AHO, but in situations where AHO is confirmed, the serum CRP performed on initial evaluation can serve as the baseline value for sequential monitoring." They recommend against using serum PCT. In terms of ESR, they comment that the ESR is no longer used routinely to diagnose AHO in children. "ESR combined with CRP may slightly improve sensitivity and negative predictive value for the diagnosis of AHO, but specific thresholds and the overall clinical utility of using both CRP and ESR for diagnostic purposes remain uncertain" (Woods et al., 2021).

"There are no data to support a particular frequency of CRP monitoring during the course of AHO in children. Measurement every 2 to 3 days during the early therapeutic course, rather than daily, followed by weekly or other periodic measurement until normalization (or a clear trend toward normalization is evident) is an acceptable approach" (Woods et al., 2021).

Government of British Columbia

The government of British Columbia provides practitioner and professional guidelines, including a guideline on CRP and ESR. Within this guideline, they provide key recommendations:

- "CRP is the preferred first test to support a diagnosis of inflammatory or infectious conditions, rather than ESR. There is no indication for ordering ESR when CRP is elevated.
- According to the British Columbia Laboratory Services Outpatient Payment Schedule, ESR will be performed only if a written indication is provided on the requisition. If CRP and ESR are ordered together, most outpatient laboratories will only perform CRP because only CRP is payable.1
- Clinical features that together **may** prompt a requisition for CRP are:
    - **unexplained symptoms** or a deterioration of health status; **and**
    - an inflammatory or infectious disease is suspected; **and**
    - a specific diagnosis is not made effectively by other means.
- **Repeat testing for CRP depends on the clinical status of the patient**. It may be used in routine monitoring of patients with inflammatory arthritis and other rheumatic conditions. For most

infections, repeat CRP is not indicated and assessment should be made on clinical grounds (e.g., when following treatment of cellulitis,2 pneumonia or urinary tract infections).

- The only indication for CRP assessment in **asymptomatic** individuals is in the stratification of cardiovascular risk. High sensitivity (hs) CRP is one of several tools which may be used in patients at intermediate cardiovascular risk to help decide whether a statin should be started. If hsCRP is desired, it should be specifically requested on the laboratory requisition" (Government of British Columbia, 2023)

Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration

Testing of serum acute phase reactants and ESR is performed in laboratories meeting Clinical Laboratory Improvement Act (CLIA) quality standards. The FDA has approved multiple tests for human CRP, including assays for conventional CRP, high-sensitivity CRP (hsCRP), and cardiac CRP (cCRP). On September 22, 2005, the FDA issued guidelines concerning the assessment of CRP (FDA, 2005). A search of the FDA Medical Devices database (FDA, 2018) on April 20, 2021, shows that the FDA has approved ESR systems from multiple companies, including the ESR Control -M Hematology Erythrocyte Sedimentation system (K972172) and the ESR Control -HC Hematology Erythrocyte Sedimentation system (K972170) by R & D Systems, the Seditainer Erythrocyte Sedimentation Rate System (K953994) from Becton Dickinson Vacutainer Systems, the Westergren Dispette for ESR (K831195) by Ulster Scientific, and the Dade ESR Kit (K823368) from American Dade.

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
85651	Sedimentation rate, erythrocyte; non-automated
85652	Sedimentation rate, erythrocyte; automated
86140	C-reactive protein
86141	C-reactive protein; high sensitivity (hsCRP)

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

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Revision History

Revision Date	Summary of Changes
09/04/2024	Reviewed and Updated: Updated background, guidelines, and evidence-based scientific references. Literature review necessitated the following changes in coverage criteria:

	Updated Note 1 for clarity. Previously read: "Coverage of CRP, ESR, CRP or ESR, or both CRP and ESR is designated based on the diagnosed or suspected inflammatory condition. Either conventional or high-sensitivity CRP testing are allowed methods of testing for CRP levels. When either CRP or ESR are allowed, CRP is the preferred biomarker." In the table within Note 1, fixed spelling of spondyloarthritis. Updated Large Vessel Vasculitis from test preference from "CRP or ESR" to "CRP and ESR", updated frequency from "NS" to "To confirm diagnosis; every 1–3 months during the first year; every 3–6 months thereafter". GCA is one of the two disorders that falls within the umbrella of large vessel vasculitis. With the updates to test preference and frequency, GCA and LVV are now the same, results in the deletion of GCA as its own separate row within Table 1. For full clarity, changed the title of the condition within that column from LVV to "Large Vessel Vasculitis (Giant Cell Arteritis, Takayasu Arteritis)"

Helicobacter pylori Testing

Policy Number: AHS – G2044 – *Helicobacter pylori* Testing	Prior Policy Name and Number, as applicable:
Initial Presentation Date: 03/19/2015 Revision Date: 03/06/2024	

Policy Description

Helicobacter pylori (*H. pylori*) is a spiral-shaped, gram-negative bacteria that thrives while living in acidic environments, growing in close association with the stomach lining. H. *pylori* infection causes chronic inflammation (infection) in the stomach and is associated with conditions such as peptic ulcer disease, chronic gastritis, gastric adenocarcinoma, and gastric mucosa associated lymphoid tissue (MALT) lymphoma (Lamont, 2023).

Related Policies

Policy Number	Policy Title
	Not applicable

Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) For individuals 18 years of age and older, urea breath testing **or** stool antigen testing to diagnose an *H. pylori* infection **MEETS COVERAGE CRITERIA** in **any** of the following situations:
   a) For individuals with dyspeptic symptoms.

b) For individuals with active peptic ulcer disease (PUD).

c) For individuals with past PUD without *H. Pylori* history.

d) For individuals with low-grade gastric mucosa-associated lymphoid tissue (MALT) lymphoma.

e) For individuals with a history of endoscopic resection of early gastric cancer (EGC).

f) For individuals with gastric intestinal metaplasia (GIM).

g) For individuals with uninvestigated dyspepsia who are under the age of 60 years and without alarm features.

h) For individuals initiating chronic treatment with a non-steroidal anti-inflammatory drug (NSAID).

i) For individuals with unexplained iron deficiency anemia.

j) For the evaluation of individuals with chronic immune thrombocytopenic purpura (ITP) and suspected *H. pylori* infection.

k) For individuals with a family history of gastric cancer.

l) For individuals who are first-generation immigrants from a high prevalence area.

2) For individuals 18 years of age and older, urea breath testing **or** stool antigen testing to measure the success of eradication of *H. pylori* infection (follow-up measurement at least 4 weeks post-treatment) **MEETS COVERAGE CRITERIA** in **any** of the following situations:

   a) For individuals with an *H. pylori*-associated ulcer.

   b) As part of the follow-up for individuals with persistent symptoms of dyspepsia following appropriate antibiotic treatment for *H. pylori*.

   c) For individuals with Gastric MALT Lymphoma.

   d) For individuals who have undergone resection of early gastric cancer.

3) For individuals 18 years of age and older undergoing endoscopic examination or who have alarm symptoms, a biopsy-based endoscopic histology test and **either** a rapid urease test **or** a culture with susceptibility testing to diagnose an *H. pylori* infection **MEETS COVERAGE CRITERIA**.

4) For individuals less than 18 years of age, urea breath **testing or** stool antigen testing to diagnose an *H. pylori* infection **MEETS COVERAGE CRITERIA** in **any** of the following situations:

   a) For individuals with chronic ITP and suspected *H. pylori* infection.

   b) To measure the success of eradication of *H. pylori* infection (follow-up measurement at least 4 weeks post-treatment).

5) For individuals less than 18 years of age, a biopsy-based endoscopic histology test and **either** a rapid urease test **or** a culture with susceptibility testing to diagnose an *H. pylori* infection **MEETS COVERAGE CRITERIA** in **any** of the following situations:

   a) For individuals with gastric or duodenal ulcers.

   b) For individuals with refractory iron deficiency anemia (when other causes have been ruled out).

6) Urea breath testing **or** stool antigen testing to diagnose an *H. pylori* infection **DOES NOT MEET COVERAGE CRITERIA** for **any** of the following situations:

a) For asymptomatic individuals of all ages.

b) For individuals 18 years and older with typical symptoms of gastroesophageal reflux disease (GERD) who do not have a history of peptic ulcer disease (PUD).

7) For individuals of all ages, serologic testing for *H. pylori* infection **DOES NOT MEET COVERAGE CRITERIA**.

8) For individuals less than 18 years of age, a biopsy-based endoscopic histology test and a rapid urease test **or a** culture with susceptibility testing to diagnose an *H. pylori* infection **DOES NOT MEET COVERAGE CRITERIA** in **any** of the following situations:

a) For children with functional abdominal pain.

b) As part of an initial investigation in children with iron deficiency anemia.

c) When investigating causes of short stature.

9) For individuals with recent use of antibiotics, proton pump inhibitors (PPIs), or bismuth, the urea breath test, stool antigen, **or** biopsy-based testing to diagnose an *H. pylori* infection **DOES NOT MEET COVERAGE CRITERIA**.

10) To diagnose an *H. pylori* infection, concurrent testing with **any** combination of the urea breath test, stool antigen testing, **and/or** biopsy-based testing **DOES NOT MEET COVERAGE CRITERIA**.

11) Nucleic acid testing for *H. pylori* **DOES NOT MEET COVERAGE CRITERIA**.

Table of Terminology

Term	Definition
ACG	American College of Gastroenterology
AGA	American Gastroenterological Association
ASCP	American Society for Clinical Pathology
ASH	American Society of Hematology
CAG	Canadian Association of Gastroenterology
CLIA '88	Clinical Laboratory Improvement Amendments of 1988
CMS	Centers for Medicare and Medicaid
DNA	Deoxyribonucleic acid
EAGEN	European Association for Gastroenterology, Endoscopy and Nutrition
EGC	Early gastric cancer
EIA	Enzyme immunoassay
ELISA	Enzyme-linked immunosorbent assay
ESNM	European Society of Neurogastroenterology and Motility
ESPGHAN	European Society for Pediatric Gastroenterology Hepatology and Nutrition
FDA	Food and Drug Administration
FIA	Fluorescence immunoassay
GERD	Gastroesophageal reflux disease
GIM	Gastric intestinal metaplasia

gyrA	Deoxyribonucleic acid gyrase subunit A
HpSA-LFIA	*Helicobacter pylori* stool antigen lateral flow immunochromatography assay
H. pylori	*Helicobacter pylori*
HP	*Helicobacter pylori*
ID	Iron deficiency
IDA	Iron deficiency anemia
IgG	Immunoglobulin G
ITP	Immune thrombocytopenic purpura
LDTs	Laboratory-developed tests
MALT	Mucosa associated lymphoid tissue
NAFLD	Non-alcoholic fatty liver disease
NASPGHAN	North American Society for Pediatric Gastroenterology, Hepatology and Nutrition
NGS	Next-generation sequencing
NICE	National Institute for Health and Care Excellence
NSAID	Non-steroidal anti-inflammatory drug
OR	Odds ratio
PCR	Polymerase chain reaction
Pg	Pepsinogen
PLA	Proprietary laboratory analyses
PPI	Proton pump inhibitor
PUD	Peptic ulcer disease
qPCR	Quantitative polymerase chain reaction
RNA	Ribonucleic acid
rRNA	Ribosomal ribonucleic acid
RUT	Rapid urease test
SA	Stool antigen
SAT	Stool antigen test
UBT	Urea breath test
USS	Updated Sydney system

Scientific Background

Infection with *H. pylori* is common, with conservative estimates at 50% of the world's population affected. Prevalence in the United States is significant, estimated to be 30 – 40% in the general population (Siao & Somsouk, 2014). *H. pylori* is associated with many conditions, such as peptic ulcer disease, chronic gastritis, and gastric mucosa associated lymphoid tissue (MALT) lymphoma. Other conditions such as dyspepsia have been attributed to *H. pylori* as well (Lamont, 2023). Common symptoms of these conditions include gastritis, dyspepsia, heartburn, and stomach pain (Jensen, 2023; Longstreth, 2022).

Identification of *H. pylori* infection is accomplished with one or more of the several tests available. The choice of test is guided by the reason for the test, cost and availability of the test, the patient's age and clinical presentation, prevalence in a population, and the patient's use of certain medications. Testing for *H. pylori* infection is done for two main reasons; to detect an active infection that will be treated and to

confirm eradication of the infection post-treatment. Invasive and non-invasive approaches have been used. Endoscopy and collection of biopsy specimens for evaluation of *H. pylori* infection and early gastric cancer detection typically is done in older individuals and those with "alarm" symptoms, including bleeding, unexplained anemia, unexplained weight loss, progressing dysphagia, recurrent vomiting, a family history of gastrointestinal cancer, or a personal history of esophagogastric malignancy. Tissue samples can be tested for *H. pylori* via methods such as a rapid urease test, culture, or staining. Molecular methods include PCR and next-generation sequencing, and serological methods include ELISA, immunoassays, and dried blood spots. Other non-invasive methods include urea breath test and stool antigen test. Testing for eradication of infection may be performed with the same tests used for diagnosis (Lamont, 2023).

Analytical Validity

Non-invasive options for detection of active *H. pylori* infection include urea breath tests and stool antigen testing. The stool antigen test is an immunoassay that detects the presence of *H. pylori* in a stool sample. The test is reported to have greater than 90% sensitivity and specificity for detection of active *H. pylori* infection, and its use has been FDA cleared for all ages. This test may be used for initial diagnostic purposes and for post-treatment testing. Urea breath tests, which take advantage of the bacteria's urease activity, may also be used to detect active *H. pylori* infection. The patient ingests a solution containing either ^{13}C or ^{14}C labeled urea, after a set amount of time, the patient's breath is collected and analyzed for the presence of ^{13}C or ^{14}C labeled CO_2. If *H. pylori* is present, it will have metabolized the labeled urea and labeled CO_2 will be detected, thus indicating infection with *H. pylori*. This test takes approximately 15-20 minutes (Lamont, 2023).

ELISA-based serological tests are also available for detection of *H. pylori*. However, serological tests often need validation at the local level, which may not be practical in routine practice. Furthermore, serological tests do not distinguish between past and present infections. Serological tests also have a very low positive predictive value in populations with low or average prevalence, as the antibodies will be detected even after an infection has been treated or naturally resolved. In these low-prevalence areas, a positive serological test is more likely to be a false positive (Lamont, 2023).

Other tests such as PCR-based tests are infrequently used. The PCR test, despite its high accuracy, is often too expensive for routine use. In fact, nested PCR tests have approached 100% sensitivity and 100% specificity for detection of *H. pylori* (Singh et al., 2008), but the test may not be widely available and may be of limited use due to high cost (Lamont, 2023; Patel et al., 2014). PCR tests have been used for diagnostic purposes as well as identifying genetic variants of the bacteria and pathogenic genes present in a patient. A variety of body fluids, such as stool and saliva, have been used in PCR tests for this bacterial species (Patel et al., 2014).

Some medications are known to inhibit the growth or urease activity of *H. pylori* and can cause a false negative *H. pylori* test result. Proton pump inhibitors, antibiotics, and bismuth-containing medications may decrease sensitivity of tests, thereby increasing rates of a false negative. Eradication testing is often done weeks after treatment is completed (Lamont, 2023).

Dechant et al. (2020) evaluated the accuracy of various rapid urease tests (RUTs) and compared it with histopathology results. No differences were detected in the sensitivity or specificity of the various RUTs and RUTs had comparable results to histology; however, in patients treated with proton pump inhibitors and antibiotics. RUTs seemed to be more sensitive compared to histology. Pohl et al. (2019) discuss the drawbacks of RUTs, including false negative test results if the bacterial load is less than 10^4 in the gastric

biopsy and false positive test results with some urease positive bacteria, affecting the sensitivity and specificity of RUTs. Commercially available RUTs, such as HpFast, CLOTest, and HpOne, have reported specificities ranging from 95% to 100%, but their sensitivity is moderate (85% to 95%) (Pohl et al., 2019).

Hussein et al. (2021) compared the sensitivity, specificity, positive, and negative predictive values of invasive tests (RUT and gastric tissue culture) and noninvasive tests (^{14}C-Urea breath test (^{14}C-UBT), stool antigen test, and CagA-IgG serology) to the gold standard quantitative PCR (qPCR) tests for *H. pylori* in Iraq. One hundred and fifteen participants strongly suspected of *H. pylori* infection were tested. Overall, the prevalence rates ranged from 47.8% to 70.4% depending on the test method. "The ^{14}C-UBT showed the highest overall performance with 97.5% sensitivity, 97% specificity, and total accuracy of 97.3% followed by SAT, RUT, Cag-IgG, and culture method." SAT had a sensitivity of 95.0% and a specificity of 91.2%. RUT had a sensitivity of 93.8% and a specificity of 94.1%. CagA-IgG had a sensitivity of 75.3% and a specificity of 85.3%. Gastric tissue culture had a sensitivity of 67.9% and a specificity of 79.4%. The authors conclude that ^{14}C-UBT "may be recommended as first choice due to its higher performance compared to other methods" (Hussein et al., 2021). Hassan et al. (2021) compared the accuracy, specificity, and sensitivity of the stool antigen test and the urea breath test in 45 children who underwent osophagogastroduodenoscopy between 2013 and 2019 in Sulaymaniyah City, Iraq. Histopathological findings from biopsies were used as a confirmatory diagnosis tool. The authors found that "UBT has a statistical significant correlation with result of biopsy, also it is more accurate and more sensitive than SAT, but they share same positive predictive value and same specificity." The authors conclude that UBT is preferred over SAT in children above six years (Hassan et al., 2021).

Abdelmalek et al. (2022) evaluated the accuracy and utility assurance of *H. pylori* stool antigen lateral flow immunochromatography assay (HpSA-LFIA) in Egypt. The study used stool samples from 200 gastric patients and compared HpSA-LFIA results to the monoclonal antibody-based ELISA kit. The authors report that HpSA-LFIA achieved sensitivity of 93.75%, specificity of 59.76%, a negative predictive value of 98.00%, positive predictive value of 31.25%, and accuracy of 65.31%. The authors conclude that "HpSA-LFIA was not accurate enough to be the sole test for diagnosis and needs other confirmatory tests in case of positive conditions" (Abdelmalek et al., 2022).

Clinical Utility and Validity

The stool antigen test has been shown to have strong accuracy. A meta-analysis by Gisbert et al. (2006) focusing on 2499 patients of 22 studies found the diagnostic test to have a sensitivity of 0.94 and a specificity of 0.97. The monoclonal version of the test was shown to be more sensitive than the polyclonal one (0.95 vs 0.83). The authors also evaluated the diagnostic test after eradication of the bacteria in 957 patients of 12 studies. The authors evaluated the antigen test at 0.93 sensitivity and 0.96 specificity post-eradication (Gisbert et al., 2006).

A new automated LIAISON® Meridian *H. pylori* SA assay, a chemiluminescent immunoassay that uses novel monoclonal antibodies for capture and detection of the *H. pylori* stool antigen, was evaluated for its clinical performance. Opekun et al. (2020) studied the utility of this assay on 277 patients who tested positive for H. pylori infection from an endoscopy. Comparing histology, culture, and rapid urease test results, the assay delivered a sensitivity of 95.5% and specificity of 97.6%. The authors conclude that LIAISON® "brings reliable noninvasive testing for *H. pylori* to the laboratory that is in very good agreement with the current, more invasive biopsy-based methods such as histology, culture, or rapid urease test" (Opekun et al., 2020).

The rapid in-office, monoclonal test is widely used and provides significant benefit in terms of availability and speed. However, a study using the test as a reference to compare against a new test found the in-office test to only have a 0.50 sensitivity and 0.96 specificity out of 162 patients (Korkmaz et al., 2015).

The UBT has also been well-validated. A meta-analysis by Ferwana et al. (2015) including 3999 patients of 23 studies found the diagnostic test to have a pooled sensitivity of 0.96 and a pooled specificity of 0.93. The authors noted that their populations had significant heterogeneity but concluded that the UBT had high diagnostic accuracy for detecting an *H. pylori* infection (Ferwana et al., 2015). This test is often considered the gold standard for diagnosing an *H. pylori* infection (Patel et al., 2014).

Serological tests to assess infection have also been used. A meta-analysis by Loy et al. (1996) focused on commercial serological kits assessing *H. pylori*. Loy et al. (1996) found these kits to have a pooled sensitivity of 0.85 and specificity of 0.79. The authors concluded that there was no major difference in accuracy between any of the kits tested (Loy et al., 1996).

As costs of sequencing decreases, use of Next Generation Sequencing (NGS) to detect *H. pylori* infection and its antibiotic resistance has increased. In a study by Nezami et al. (2019), 133 H. *pylori* positive specimens from histological evaluation were analyzed by NGS to detect mutations in gyrA, 23S rRNA, and 16s rRNA genes. NGS detected H. *pylori* in 126/133 cases (95% sensitivity). NGS also detected multiple mutations associated with resistance in 92 cases (73%), one mutation in 63 cases (50%), and mutations in several genes in 29 cases (23%). In the 58 cases where treatment history was available, therapy failure was observed in cases where the number of mutated genes was high. Therapy failed in 11/16 cases with multiple gene mutations and 5/27 cases with one gene mutation (Nezami et al., 2019).

Yang et al. (2019) performed a meta-analysis investigating the association between *H. pylori* and colorectal cancer. Twenty-seven studies encompassing 14357 cases were included. The authors found an increased rate of colorectal cancer with *H. pylori* infection (odds ratio [OR] = 1.27). The authors also identified odds ratios for certain subgroups, such as Western countries (OR = 1.34), serological testing (OR = 1.20), multiple methods of testing (OR = 2.63), and cross-sectional studies (OR = 1.92) (Yang et al., 2019).

Wang et al. (2019) performed a meta-analysis assessing the association between *H. pylori* and osteoporosis. Twenty-one studies totaling 9655 patients were analyzed. The authors found that *H. pylori* infection was associated with an increased risk of osteoporosis with an odds ratio of 1.39. However, the decrease of bone mineral density in *H. pylori* positive patients was not found to be significant compared to *H. pylori* negative patients (Wang et al., 2019).

Zhou et al. (2019) investigated the association between *H. pylori* infection and non-alcoholic fatty liver disease (NAFLD). Fifteen studies including 97228 patients were evaluated. The authors identified an increased risk of NAFLD in *H. pylori* positive patients compared to *H. pylori* negative patients by an odds ratio of 1.19. Similar results were found despite differing subgroups, such as geographical locations. Testing method did not significantly change the results, and there was no significant difference when using multiple detection methods (Zhou et al., 2019).

Halland et al. (2021) assessed two novel enzyme assays (EIA), H. PYLORI QUIK CHEK™ and H. PYLORI CHEK™, for the detection of *H. pylori* antigen in stool from 271 patients in America, Germany, and Bangladesh. The EIA results were compared to clinical diagnosis, which included histological analysis and rapid urease test. H. PYLORI QUIK CHEK™ had a sensitivity of 92% and a specificity of 91%. H. PYLORI

CHEK™ had a sensitivity of 91% and a specificity of 100%. The authors concluded that "the H. PYLORI QUIK CHEK™ and H. PYLORI CHEK™ assays demonstrate excellent clinical performance compared the composite reference method" (Halland et al., 2021). Marrero Rolon et al. (2022) have developed and tested a real-time PCR assay to simultaneously detect *H. pylori* infection and genotypic markers of clarithromycin resistance. *H. pylori* infection can be treated with clarithromycin-based therapy; The American College of Gastroenterology (ACG) recommends clarithromycin-based triple therapy as first-line treatment in regions where clarithromycin resistance is known to be below 15% in patients with no history of macrolide exposure. "Clarithromycin resistance is most commonly caused by point mutations in the 23S rRNA (rRNA) gene, including A2143G, A2142G, and A2142C, which result in decreased macrolide binding to the 23S rRNA ribosomal subunit; clarithromycin resistance is considered the main cause of clarithromycin therapy failure." The authors tested 524 stool samples. *H. pylori* stool antigen tests were used as a control test for *H. pylori* detection. Sanger sequencing was used as control tests for genetic susceptibility. PCR results were positive for 98% of positive antigen stool tests. "The clarithromycin-based triple therapy success was lower when resistance was predicted by PCR (41%) than when no resistance was predicted (70%; P = 0.03)." The authors conclude that the PCR assay can diagnose *H. pylori* infection and provide genetic susceptibility information. The authors suggest the need for susceptibility-guided therapy when clarithromycin-based therapy is considered (Marrero Rolon et al., 2022).

Nguyen Wenker et al. (2023) studied the predictive performance of current guidelines about *H. pylori* testing in the United States. The authors investigated the association between *H. pylori* presence and *H. pylori* risk factors. *H. pylori* presence was determined based on histopathology, stool antigen, urea breath test, immunoglobulin G serology, or prior treatment. The risk factors were selected based on the Houston Consensus Conference and American College of Gastroenterology guidelines. The study included 942 patients undergoing upper endoscopy with gastric biopsies for any indication from one hospital in Houston, Texas. Overall, the risk factors with the highest predictive performance were "first-generation immigrant" and "Hispanic or black race/ethnicity." The other seven risk factors included had low predictive values. The authors concluded that "the performance of individual risk factors identified by the Houston Consensus Conference and ACG was generally low for predicting H pylori infection except for black or Hispanic race/ethnicity and first-generation immigrant status" (Nguyen Wenker et al., 2023).

Guidelines and Recommendations

American Gastroenterological Association (AGA)

The AGA recommends that "patients 55 years or younger without alarm features should receive *H. pylori* test and treat followed by acid suppression if symptoms remain" and note that "*H. pylori* testing is optimally performed by a 13C-urea breath test or stool antigen test." Alarm features include symptoms such as recurrent vomiting and weight loss. Additionally, the AGA indicates that "although the yield of endoscopy is low, it is recommended for patients older than 55 years of age and for younger patients…presenting with new-onset dyspepsia." They reason that endoscopy with biopsy is the preferred test for this age group because upper gastrointestinal malignancy becomes more common after age 55 years (Talley, 2005).

In 2015 the AGA published a technical review on Upper Gastrointestinal biopsy to evaluate dyspepsia in the absence of visible mucosal lesions and found that:

- In the defined population, biopsy of normal-appearing gastric mucosa can detect HP [*H. pylori*] infection that would be missed on the exam without biopsies. The quality of evidence is very low, and there are noninvasive methods to detect HP infection.
- "Detection of HP infection with tissue biopsy and its eradication in patients with dyspepsia is associated with symptom improvement and reduction of risk for HP-related comorbidities, including gastric cancer compared with no biopsy (or no eradication). The quality of evidence is moderate. The effect on symptom resolution is not universal and it does not appear to improve well-being. Quality of evidence for this statement is low" (Allen et al., 2015).

The AGA also released guidelines focusing on gastric intestinal metaplasia. In it, they recommend testing for *H. pylori* (followed by eradication) over no testing and eradication (Gupta et al., 2020).

The AGA released guidelines on gastrointestinal evaluation of iron deficiency anemia. AGA recommends that patients with iron deficiency anemia, without other identifiable etiology after bidirectional endoscopy, should undergo noninvasive testing for H. *pylori* over no testing at all to reduce the incidence of gastric cancer (Ko et al., 2020).

American College of Gastroenterology/Canadian Association of Gastroenterology

The ACG and CAG have released guidelines on testing for *H. pylori*:

- All patients with active peptic ulcer disease (PUD), a past history of PUD (unless previous cure of *H. pylori* infection has been documented), low-grade gastric mucosa-associated lymphoid tissue (MALT) lymphoma, or a history of endoscopic resection of early gastric cancer (EGC) should be tested for *H. pylori* infection. Those who test positive should be offered treatment for the infection.
- In patients with uninvestigated dyspepsia who are under the age of 60 years and without alarm features, non-endoscopic testing for *H. pylori* infection is a consideration. Those who test positive should be offered eradication therapy.
- When upper endoscopy is undertaken in patients with dyspepsia, gastric biopsies should be taken to evaluate for *H. pylori* infection. Infected patients should be offered eradication therapy.
- Patients with typical symptoms of gastroesophageal reflux disease (GERD) without history of PUD need not be tested for *H. pylori* infection. For those who are found to be infected, treatment should be offered, acknowledging that effects on GERD symptoms are unpredictable.
- In patients taking long-term low-dose aspirin, testing for *H. pylori* infection could be considered.
- Patients initiating chronic treatment with a non-steroidal anti-inflammatory drug (NSAID) should be tested for *H. pylori* infection. Those who test positive should be offered eradication therapy.
- Patients with unexplained iron deficiency (ID) anemia despite an appropriate evaluation or idiopathic thrombocytopenic purpura should be tested for *H. pylori* infection.
- There is insufficient evidence to support routine testing and treating of *H. pylori* in asymptomatic individuals with a family history of "gastric cancer or patients with lymphocytic gastritis, hyperplastic gastric polyps and hyperemesis gravidarum".
- The ACG recommends the breath test and fecal stool antigen test as eradication tests, supported by moderate evidence (Chey et al., 2017).

Another set of joint guidelines from the ACG and Canadian Association of Gastroenterology (CAG) noted that dyspepsia patients under 60 should be tested for *H. pylori* (Moayyedi et al., 2017).

National Institute for Health and Care Excellence (NICE)

NICE recommends testing for *H. pylori* with a carbon-13 urea breath test or a stool antigen test. A re-test should be with a breath test. Office-based serological tests are not recommended. NICE recommends a "2-week washout period after proton pump inhibitor (PPI) use before testing for *Helicobacter pylori*." NICE recommends that individuals with positive *H. pylori* tests be offered therapy to eradicate the bacteria; however, they note that re-testing to confirm eradication should not be routinely offered. NICE limits the recommendation for post-treatment testing to **"**people with peptic ulcer (gastric or duodenal)…6 to 8 weeks after beginning treatment, depending on the size of the lesion" (NICE, 2019).

NICE released further guidelines in 2015 reaffirming the carbon-13 urea breath test and the stool antigen test to test for *H. pylori*. A locally validated lab-based serology test may also be used to assess *H. pylori*. NICE reaffirms the two week washout period before testing for *H. pylori* if the patient is on PPIs as well as the four week washout period if the patient is on antibiotics (NICE, 2015).

American College of Cardiology

The American College of Cardiology recommends testing for and eradicating *H. pylori* in patients with a history of ulcer disease before starting chronic antiplatelet therapy (Bhatt et al., 2008).

World Gastroenterology Organization

The World Gastroenterology Organization Global Guidelines on *Helicobacter pylori* recommends testing for *H. pylori* based on evidence-based indications, noting that these indications may differ in different regions of the world based on prevalence, resources, competing needs, and individual patient factors. The guidelines state that "peptic ulcer disease is the prime indication in most of the world." The guidelines list other indications for the treatment of *H. pylori* as: past or present duodenal and/or gastric ulcer (with or without complications), gastric MALT lymphoma, gastric mucosal atrophy and/or intestinal metaplasia, resection of gastric cancer, first-degree relatives with gastric cancer, functional dyspepsia, NSAID use, before long-term aspirin therapy in patients at high risk of ulcers and ulcer-related complications, during long-term low-dose aspirin therapy in patients with a history of upper gastrointestinal bleeding and perforation, patients with gastroesophageal reflux disease who require long-term proton-pump inhibitors, as a strategy for gastric cancer prevention in communities with a high incidence, and unexplained iron-deficiency anemia or idiopathic thrombocytopenic purpura (Katelaris et al., 2023).

European Association for Gastroenterology, Endoscopy and Nutrition (EAGEN), European Society of Neurogastroenterology and Motility (ESNM), and European Society for Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN)

The pan-European guideline recommends the use of ^{13}C -urea breath tests as a noninvasive alternative for testing for "all indications of *Helicobacter pylori* testing if endoscopy is not required or if biopsies are contraindicated" and as "a preferred option for conformation of *Helicobacter pylori* eradication in adults and children." Alternatively, when there is indication for endoscopy and no contraindication for biopsy, the guidelines recommend RUT as the first-line diagnostic tests (Keller et al., 2021).

ESPGHAN and The North American Society for Pediatric Gastroenterology, Hepatology and Nutrition (NASPGHAN)

The ESPGHAN and NASPGHAN have issued updated guidelines for management of *H. pylori* in children and adolescents. They have proposed recommendations for diagnosis and management of *H. pylori* infection in pediatric patients. They have defined pediatric patients as children and adolescents below 18 years of age. The following recommendations were stated:

The guidelines recommend biopsies for rapid urease test and other cultures should only be taken if treatment is likely to be offered in the case of a confirmed infection. Treatment may be considered if *H. pylori* is an incidental finding at endoscopy.

The guidelines recommend against a "test and treat" strategy for *H. pylori* infection in children. The panelists explained that performing a noninvasive test to detect infection and treat is not needed because *H. pylori* infection usually does not cause any symptoms in the absence of peptic ulcer disease (PUD).

The guidelines recommend that "testing for *H. pylori* be performed in children with gastric or duodenal PUD."

The guidelines recommend against diagnostic testing for *H. pylori* infection in children with functional abdominal pain, iron deficiency anemia, and when investigating causes of short stature. Serology-based testing was also not recommended.

Proton pump inhibitors (PPIs) should be stopped two weeks before *H. pylori* testing, and antibiotics should be stopped four weeks before *H. pylori* testing. Diagnosis should be based on either: "positive culture or *H. pylori* gastritis on histopathology with at least 1 other positive biopsy-based test."

The non-invasive diagnostic testing was indicated in children when investigating causes of chronic immune thrombocytopenic purpura or for the assessment of anti-*H. pylori* therapy at least after four weeks of therapy (L. Jones et al., 2017).

Japanese Society for Pediatric Gastroenterology, Hepatology and Nutrition (JSPGHAN)

The JSPGHAN have updated their guidelines for *H. pylori* testing in pediatrics, including recommendations for diagnostic methods in children.

For diagnosis using endoscopic biopsy specimens, the guidelines recommend considering the performance and accuracy of the rapid urease test, recommending an additional urea breath test or stool antigen test when there is inconsistency between histopathology and the rapid urease test. The guidelines further recommend histological examination of gastric biopsies, and culture diagnostic tests to diagnose active *H. pylori* infection (Kato et al., 2020).

For diagnosis without endoscopic biopsy specimens, the guidelines recommend ^{13}C-urea breath test and stool antigen tests. To increase the diagnosis accuracy, the guidelines recommend more than two tests (two noninvasive tests or a biopsy-based and a noninvasive test) be completed. The guidelines recommend urea breath test or stool antigen test four or more weeks after treatment to confirm eradication of *H. pylori* and recommend against using endoscopic biopsy methods and single serological tests to confirm eradication. The guidelines also recommend against anti-*H. pylori* antibody tests as a single test to diagnose *H. pylori* in a clinical setting (Kato et al., 2020).

Maastricht V/Florence Consensus Report

The Maastricht V/Florence Consensus report was published in 2017 on behalf of the European Helicobacter and Microbiota Study Group and Consensus panel. The panel reports that UBT is "the most investigated and best recommended non-invasive test in the context of a 'test-and-treat strategy". The panel also notes that monoclonal tests can be used and that serological tests can be used only after validation. However, rapid "office" serology tests are not recommended and "should be avoided". The guidelines recommend the rapid urease test (RUT) as a first line diagnostic test if there is an indication for

endoscopy and no contraindication for biopsy. The guideline state that *H. pylori* is linked to "unexplained iron deficiency anaemia (IDA), idiopathic thrombocytopenic purpura, and vitamin B12 deficiency", and in these disorders, an *H. pylori* infection should be "sought and eradicated." The guidelines state that PPIs should be stopped two weeks and antibiotics and other bismuth compounds should be stopped four weeks before testing for *H. pylori*. In cases of chronic (active) gastritis in which *H. pylori* is not detected by histochemistry, immunohistochemical testing of *H. pylori* can be used as an ancillary test. If histology is normal, no immunohistochemical staining should be performed. It is recommended to perform clarithromycin susceptibility testing when a standard clarithromycin-based treatment is considered as the first-line therapy, except in populations or regions with well documented low clarithromycin resistance (<15%). Pepsinogen (Pg) serology is considered the most useful non-invasive test to explore gastric mucosa status (non-atrophic vs atrophic). The PgI/PgII ratio can never be assumed as a biomarker of gastric neoplasia. UBT is the best option for confirmation of *H. pylori* eradication and monoclonal SAT is an alternative. It should be performed at least four weeks after completion of therapy (Malfertheiner et al., 2017).

The Maastricht IV from 2012 also addressed testing for the cagA and vacA variants, stating that no specific genetic or virulence markers can be recommended at this time (Malfertheiner et al., 2012).

American Society for Clinical Pathology (ASCP)

The ASCP recommends against using the serological tests for *H. pylori* and recommends the stool antigen and breath tests instead. The ASCP states that serological evaluation is no longer clinically useful and the stool and breath tests have superior statistical power (ASCP, 2016).

American Society of Hematology (ASH)

American Society of Hematology (ASH) published an update to the immune thrombocytopenic purpura guidelines in 2019. In it, they "suggest" that "Screening for *H pylori* be considered for patients with ITP in whom eradication therapy would be used if testing is positive." However, ASH still recommends against "routine testing for *H pylori* in children with chronic ITP" (Neunert et al., 2020).

Houston Consensus Conference

This conference included 11 experts on "management of adult and pediatric patients with *H. pylori*, from different geographic regions of the United States" and was convened to "discuss key factors in diagnosis of *H. pylori* infection, including identification of appropriate patients for testing, effects of antibiotic susceptibility on testing and treatment, appropriate methods for confirmation of infection and eradication, and relevant health system considerations." Two cohorts of approval were present: one of the 11 experts, and another consisting of a selected group of United States-based gastroenterologists. These recommendations were intended to provide practical advice for US practitioners, and guidelines to be adopted by US health care systems.

Recommendations approved by both groups are listed below:

- "Statement 1: We recommend that all patients with active *H pylori* infection be treated (100% agree/strongly agree, Grade 1A).
- Statement 2: All patients with current or past gastric or duodenal ulcers should be tested for *H pylori* infection (100% agree/strongly agree; Grade 1A).
- Statement 3: We recommend that all patients with uninvestigated dyspepsia be tested for *H pylori* infection (100% agree/strongly agree, Grade 1A).

- Statement 4: We recommend routine testing for *H pylori* infection in patients with reflux symptoms only if they are at high risk for *H pylori*-related disease (91% agree/strongly agree, Grade 1C).
- Statement 5: We recommend that patients with gastric mucosa-associated lymphoid tissue (MALT) lymphoma be tested for *H pylori* infection (100% agree/strongly agree, Grade 1B).
- Statement 6: We recommend that individuals with family history of gastric cancer be tested for *H pylori* infection (100% agree/strongly agree, Grade 1B).
- Statement 7: We recommend that patients who are first-generation immigrants from high prevalence areas be tested for *H pylori* infection (82% agree/strongly agree, Grade 1B).
- Statement 8: We suggest that patients of Latino and African American racial or ethnic groups may be considered for *H pylori* testing due to their high risk of infection (91% agree/strongly agree, Grade 2C)."
- Statement 17: We recommend that validated diagnostic testing of stool or gastric mucosal biopsy by culture and susceptibility, or molecular analysis be universally available (100% agree/strongly agree, Grade 1)
- Statement 18: We suggest that antibiotics that may be routinely evaluated for susceptibility include amoxicillin, clarithromycin, levofloxacin, metronidazole, and tetracycline (100% agree/strongly agree, Grade 2C).
- Statement 20: We recommend the use of tests for active *H pylori* infection (ie, UBT, HpSAg testing) for the initial diagnosis (100% agree/strongly agree, Grade 1A).
- Statement 22: We recommend that serology not be utilized for detection of active *H pylori* infection (100% agree/strongly agree, Grade 1A).
- Statement 23: We recommend that bismuth and antibiotics be stopped at least 4 weeks before *H pylori* testing with tests for active infection (ie, UBT, and HpSAg testing and histology; 100% agree/strongly agree, Grade 1C).
- Statement 27: We recommend that all patients receiving treatment for *H pylori* receive posttreatment confirmation of eradication. We recommend that only tests that evaluate for active infection, such as UBT, HpSAg test, or histology (if endoscopy is required for other reasons), are utilized for this purpose (100% agree/strongly agree, Grade 1A).
- Statement 28: Once appropriate testing has confirmed eradication, we recommend against further *H pylori* testing, (100% agree/strongly agree, Grade 1C)"

The following recommendations reached a consensus by the expert panel, but not the external group:

- "Statement 9: We recommend that patients with idiopathic thrombocytopenia be tested for *H pylori* infection (experts vs survey: 100% vs 68% agree/strongly agree, Expert Grade 1B)
- Statement 10: We suggest that patients receiving long-term PPIs (>1 month) be tested for *H pylori* infection (experts vs survey: 82% vs 68% agree/strongly agree, Expert Grade 2C)
- Statement 11: We recommend that family members residing in the same household of patients with proven active *H pylori* infections undergo *H pylori* testing (experts vs survey: 91% vs 78% agree/strongly agree, Expert Grade 1B)
- Statement 12: We recommend that individuals with a family history of peptic ulcer disease be tested for *H pylori* infection (experts vs survey: 91% vs (73% agree/strongly agree, Expert Grade 1B)" (El-Serag et al., 2018).

Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations

(NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

On Feb 22, 2012, the FDA approved the BreathTek UBT for H. pylori Kit created by Otsuka America Pharmaceutical, Inc. TheBreathTek UBT for H. pylori Kit (BreathTek UBT Kit) is intended for use in the qualitative detection of urease associated with H. pylori in the human stomach and is indicated as an aid in the initial diagnosis and post-treatment monitoring of H. pylori infection in adults, and pediatric patients three to 17 years old. The test may be used for monitoring treatment if used at four weeks following completion of therapy. The FDA notes its sensitivity and specificity to be 0.958 and 0.992 respectively (FDA, 2012).

On Jan 17, 2002, the FDA approved the BreathTek UBiT for H. pylori created by Meretek Diagnostics Inc. The scientific basis underlying the BreathTek UBT and the BreathTek UBiT UBT kit is identical. The urea breath test is FDA cleared for use in individuals 18 years of age and older (FDA, 2002).

On February 18, 2020, the FDA approved the PyloPlus UBT System created by ARJ Medical Inc. PyloPlus detects urease associated with H. pylori in the stomach and is indicated as an aid in the initial diagnosis of H. pylori infection in adults 18 years and older (FDA, 2023).

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
83009	Helicobacter pylori, blood test analysis for urease activity, non-radioactive isotope (eg, C-13)
83013	Helicobacter pylori; breath test analysis for urease activity, non-radioactive isotope (eg, C-13)
83014	Helicobacter pylori; drug administration
86318	Immunoassay for infectious agent antibody(ies), qualitative or semiquantitative, single step-method (eg, reagent strip);
86677	Antibody; Helicobacter pylori
87070	Culture, bacterial; any other source except urine, blood or stool, aerobic, with isolation and presumptive identification of isolates
87077	Culture, bacterial; aerobic isolate, additional methods required for definitive identification, each isolate
87081	Culture, presumptive, pathogenic organisms, screening only;
87149	Culture, typing; identification by nucleic acid (DNA or RNA) probe, direct probe technique, per culture or isolate, each organism probed

87150	Culture, typing; identification by nucleic acid (DNA or RNA) probe, amplified probe technique, per culture or isolate, each organism probed
87153	Culture, typing; identification by nucleic acid sequencing method, each isolate (eg, sequencing of the 16S rRNA gene)
87181	Susceptibility studies, antimicrobial agent; agar dilution method, per agent (eg, antibiotic gradient strip)
87186	Susceptibility studies, antimicrobial agent; microdilution or agar dilution (minimum inhibitory concentration [MIC] or breakpoint), each multi-antimicrobial, per plate
87205	Smear, primary source with interpretation; Gram or Giemsa stain for bacteria, fungi, or cell types
87338	Infectious agent antigen detection by immunoassay technique, (eg, enzyme immunoassay [EIA], enzyme-linked immunosorbent assay [ELISA], fluorescence immunoassay [FIA], immunochemiluminometric assay [IMCA]) qualitative or semiquantitative; Helicobacter pylori, stool
87339	Infectious agent antigen detection by immunoassay technique, (eg, enzyme immunoassay [EIA], enzyme-linked immunosorbent assay [ELISA], fluorescence immunoassay [FIA], immunochemiluminometric assay [IMCA]) qualitative or semiquantitative; Helicobacter pylori
88305	Level IV - Surgical pathology, gross and microscopic examination - Stomach biopsy
0008U	Helicobacter pylori detection and antibiotic resistance, DNA, 16S and 23S rRNA, gyrA, pbp1, rdxA and rpoB, next generation sequencing, formalin-fixed paraffin-embedded or fresh tissue or fecal sample, predictive, reported as positive or negative for resistance to clarithromycin, fluoroquinolones, metronidazole, amoxicillin, tetracycline, and rifabutin Proprietary test: AmHPR H. Antibiotic Resistance Panel Lab/Manufacturer: American Molecular Laboratories, Inc

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

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Revision History

Revision Date	Summary of Changes
03/06/2024	Reviewed and Updated: Updated the background, guidelines and recommendations, and evidence-based scientific references. Literature review did not necessitate any modifications to coverage criteria.
03/01/2023	Reviewed and Updated: Updated the background, guidelines and recommendations, and evidence-based scientific references. Literature review did not necessitate any modifications to coverage criteria. The following edits were made for clarity: CC1 formerly had subcriteria a and b, have split into individual CC for clarity. Reorganized so that adult testing are grouped and pediatric testing are grouped, making former CC4 now CC3. Former CC5, now CC6, split into subcriteria for clarity. All other CC edited for clarity and consistency.

03/09/2022	Annual review: Updated background, guidelines and recommendations, and evidence-based scientific references. Literature review did not necessitate modification to coverage criteria. Addition of "or" to several coverage criteria for clarity.
3/3/2021	Annual review: Updated background, guidelines, and evidence-based scientific references. Literature review did necessitate the following modifications to the coverage criteria: addition per 2018 Houston Consensus Conference (El-Serag et al, 2018) of the following CC to CC1: • In patients with family history of gastric cancer • In patients who are first-generation immigrants from high prevalence areas
3/10/2020	Annual review: Updated background, guidelines, and evidence-based scientific references. Literature review did necessitate the following modifications to the coverage criteria: addition for clarity of susceptibility testing in CC3, CC4 and CC7; addition of CC1.a.vi. "in patients with gastric intestinal metaplasia (GIM)" per AGA 2019.
03/01/2019	Annual review: Updated definitions, background, guidelines, and evidence-based scientific references. • Added the word "either" to clarify CC stating that "Biopsy-based endoscopic histology test and **either** Rapid Urease Test or culture **MEETS COVERAGE CRITERIA** in pediatric patients (<18y)...." • Reworded CC concerning PCR testing for clarity to state: "The use of nucleic acid testing for *H. pylori*, including polymerase chain reaction (PCR), 16S rRNA, 23S rRNA, and next-generation sequencing (NGS) of *H. pylori*, **DOES NOT MEET COVERAGE CRITERIA** as it not practical for routine diagnosis." There is a PLA code for such testing. Added PLA code 0008U and CPT 87149, 87150 and 87153.
03/16/2018	Annual review: Definitions, Background, Guidelines and Recommendations and Evidence-based Scientific References were updated. Literature review did necessitate change in coverage criteria: CC1A: reworded and added specific conditions when testing required for adults per ACG (Chey et al, 2017); CC2: addition criteria for pediatrics since non-invasive testing is not recommended in children, only in specific situations per ESPGHAN&NASPGHAN (Jones et al, 2017); CC3 reworded from old CC3 per ACG (Chey et al, 2017) and added pediatric per SPGHAN&NASPGHAN (Jones et al, 2017); CC5: addition of biopsy-based testing as it is recommended diagnostic test strategy for pediatrics per ESPGHAN&NASPGHAN (Jones et al, 2017) CC6: addition of biopsy-based testing per ESPGHAN&NASPGHAN (Jones et al, 2017) CC7: addition of biopsy-based testing per ACG 2017 and 2007 CC8: addition per ESPGHAN&NASPGHAN (Jones et al, 2017) per ACG 2007 Tests are not reliable if patient is using those medications Added CPT Codes 87081, 87181, 87186, 87205, 88305 and 87077 as PA not required
03/20/2017	Annual review: Updated coverage criteria based on guidelines from the American Journal of Gastroenterology (2017): clarifying statements regarding coverage for urea breath testing/stool antigen (coverage criteria #1 A-I) Added clarifying statement regarding aspirin use in coverage criteria #1F.
02/26/2016	Annual review: Literature review did not necessitate change in coverage criteria.

03/19/2015	Initial presentation

Hepatitis Testing

Policy Number: AHS – G2036 – Hepatitis Testing	Prior Policy Name and Number, as applicable: • AHS-G2036-Hepatitis C
Initial Presentation Date: 11/16/2015 Effective Date: 02/01/2025	

Policy Description

Infectious hepatitis is an inflammation of the liver caused by the hepatitis viruses. Hepatitis C is a blood-borne virus that can be spread via sharing needles or other equipment to inject drugs as well as in inadequate infection control in healthcare settings (CDC, 2018). Hepatitis C causes liver disease and inflammation. A chronic HCV infection can lead to hepatic damage, including cirrhosis and hepatocellular carcinoma, and is the most common cause of liver transplantation in the United States (AASLD-IDSA, 2015).

Hepatitis B is spread by the "Percutaneous, mucosal, or nonintact skin exposure to infectious blood, semen, and other body fluids." As the hepatitis B virus is concentrated most highly in blood, "percutaneous exposure is an efficient mode of transmission", though HBV can also be transmitted through birth to an infected mother and sexual contact with an infected person and less commonly through needle-sticks or other sharp instrument injuries, organ transplantation and dialysis, and interpersonal contact through sharing items, such as razors or toothbrushes or contact with open sores of an infected person. Similar to HCV infection, 15% to 25% of people with chronic HBV infection develop chronic liver disease (CDC, 2020a).

The general route of transmission for the hepatitis A virus (HAV) is through the fecal-oral route by close person-to-person contact with an infected person, sexual contact with an infected person, or the ingestion of contaminated food or water, with the bloodborne transmission of HAV being uncommon

(CDC, 2020a). Though death is uncommon and most people with acute HAV infection recover with no lasting liver damage, HAV remains a worldwide public health issue and is endemic in many low- to middle-income countries (CDC, 2020a; Keles et al., 2021).

For HCV and HBV screening in pregnant individuals, please see AHS-G2035-Prenatal Screening (Nongenetic).

Related Policies

Policy Number	Policy Title
AHS-G2035	Prenatal Screening (Nongenetic)
AHS-G2110	Serum Marker Panels for Hepatic Fibrosis in the Evaluation and Monitoring of Chronic Liver Disease
AHS-G2157	Diagnostic Testing of Common Sexually Transmitted Infections
AHS-G2173	Gamma-glutamyl Transferase Testing in Adults

Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

Hepatitis B

1) For all individuals 18 years of age and older, triple panel testing (hepatitis B surface antigen [HBsAg], hepatitis B surface antibody [anti-HBs], total antibody to hepatitis B core antigen [anti-HBc]) for Hepatitis B (HBV) infection once per lifetime **MEETS COVERAGE CRITERIA**.

2) For asymptomatic, non-pregnant individuals, the following annual HBV infection screening **MEETS COVERAGE CRITERIA**:
   a) HBsAg and hepatitis B surface antibody (anti-HBs) for infants born from an HBsAg-positive individual.
   b) Triple panel testing (HBsAg, anti-HBs, anti-HBc) when **one** of the following high-risk situations is met:
      i) For individuals born in or who have recently traveled to geographic regions with a HBV prevalence 2% or higher (see Note 1).
      ii) For U.S.-born individuals not vaccinated as infants whose parents were born in geographic regions with an HBV prevalence 8% or higher (see Note 1).
      iii) For individuals with a history of incarceration.
      iv) For individuals infected with HIV.
      v) For individuals with a history of sexually transmitted infections or multiple sex partners.
      vi) For men who have sex with men.
      vii) For household contacts, needle-sharing contacts, and sex partners of HBV-infected individuals.

viii) For injection-drug users.

ix) For individuals with an active hepatitis C virus infection or who have a history of hepatitis C virus infection.

x) For individuals with elevated liver enzymes.

xi) For individuals who are on long-term hemodialysis treatment.

xii) For individuals with diabetes.

xiii) For healthcare and public safety workers exposed to blood or body fluids.

3) For individuals who test positive for anti-HBc, follow up IgM antibody to anti-HBc (IgM anti-HBc) testing to distinguish between an acute or chronic infection **MEETS COVERAGE CRITERIA**.

4) For the confirmation of seroconversion after hepatitis B vaccination, anti-HBs testing **MEETS COVERAGE CRITERIA**.

5) For individuals who test positive for HBV by initial antibody screening and who will undergo immunosuppressive drug therapy, HBV DNA testing **MEETS COVERAGE CRITERIA**.

Hepatitis C

6) For all individuals 18 years of age and older, antibody testing for Hepatitis C (HCV) infection once per lifetime **MEETS COVERAGE CRITERIA**.

7) For any individual with the following recognized conditions or exposures, one-time, post-exposure antibody testing for HCV infection **MEETS COVERAGE CRITERIA**:

a) For individuals who have used illicit intranasal or injectable drugs.

b) For individuals who have received clotting factor concentrates produced before 1987.

c) For individuals with a history of hemodialysis.

d) For individuals with evidence of liver disease (based on clinical presentation, persistently abnormal ALT levels, or abnormal liver function studies).

e) For individuals infected with HIV.

f) For individuals who received an organ transplant before July 1992.

g) For individuals who received a blood transfusion or blood component before July 1992.

h) For individuals notified that they received blood from a donor who later tested positive for an HCV infection.

i) For individuals with a history of incarceration.

j) For individuals who received a tattoo in an unregulated setting.

k) For healthcare, emergency medical, and public safety workers after needle sticks, sharps, or mucosal exposures to HCV-positive blood.

l) For children born from an HCV-positive individual.

m) For current sexual partners of HCV-infected persons.

8) Routine periodic antibody testing for HCV **MEETS COVERAGE CRITERIA** for individuals with **any** of the following ongoing risk factors (while risk factors persist):
    a) For individuals who currently inject drugs and share needles, syringes, or other drug preparation equipment.
    b) For individuals who are receiving ongoing hemodialysis.
    c) For individuals engaging in high-risk sexual behavior.
9) Qualitative nucleic acid testing for HCV **MEETS COVERAGE CRITERIA** in **any** of the following situations:
    a) As a follow up for individuals who test positive for HCV by initial antibody screening (to differentiate between active infection and resolved infection).
    b) One time screening for perinatally exposed infants who are 2-6 months of age.
10) Prior to the initiation of direct acting anti-viral (DAA) treatment, one time testing for HCV genotype to guide selection of the most appropriate antiviral regimen **MEETS COVERAGE CRITERIA**.
11) Testing for HCV viral load with a quantitative nucleic acid test **MEETS COVERAGE CRITERIA** in **any** of the following situations:
    a) Prior to the initiation of DAA therapy.
    b) After four weeks of DAA therapy.
    c) At the end of treatment.
    d) Twelve, twenty-four, and forty-eight weeks after completion of treatment.

Hepatitis A

12) For individuals with signs and symptoms of acute viral hepatitis and who have tested negative for HBV and HCV, testing for IgM anti-hepatitis A (HAV) or qualitative testing for HAV RNA **MEETS COVERAGE CRITERIA.**
13) Quantitative nucleic acid testing for HAV viral load **DOES NOT MEET COVERAGE CRITERIA.**

Hepatitis D

14) For individuals who have tested positive for HBV, testing for hepatitis D virus (HDV) antibody (anti-HDV) or qualitative testing for HDV RNA **MEETS COVERAGE CRITERIA.**
15) Quantitative nucleic acid testing for HDV viral load **DOES NOT MEET COVERAGE CRITERIA.**

NOTES:

Note 1: The CDC defines HBsAg prevalence by geographic region: https://wwwnc.cdc.gov/travel/yellowbook/2020/travel-related-infectious-diseases/hepatitis-b.

Table of Terminology

Term	Definition
AASLD	American Association for the Study of Liver Diseases
Ab	Antibody
AFP	Alpha fetoprotein
Ag	Antigen
AGA	American Gastroenterological Association
ALT	Alanine aminotransferase
anti-HBc	Total antibody to hepatitis B core antigen
anti-HBs	Hepatitis B surface antibody
aPTT	Activate partial thromboplastin time
AST	Aspartate aminotransferase
CBC	Complete blood count
CDC	Centers for Disease Control and Prevention
CHC	Chronic hepatitis C
CIA	Chemiluminescence immunoassays
CLIA '88	Clinical Laboratory Improvement Amendments of 1988
CMS	Centers for Medicare and Medicaid Services
DAA	Direct acting anti-viral
DBS	Dried blood spot
EASL	European Association for the Study of the Liver
EIA	Enzyme immunoassays
ELISA	Enzyme-linked immunosorbent assay
FBC	Full blood count
FDA	Food and Drug Administration
HAV	Hepatitis A virus
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HCR	Hepatitis C Screening
HCV	Hepatitis C virus
HDV	Hepatitis D (delta) virus
HIV	Human immunodeficiency virus
ICP	Intrahepatic cholestasis of pregnancy
IDSA	Infectious Disease Society of America
IgM	Immunoglobulin M
IgM anti-HBc	IgM antibody to anti-HBc
IHS	Indian Health Services
INR	International normalized ratio
LDH	Lactic acid dehydrogenase
LDTs	Laboratory-developed tests
LoE	Level of evidence

MTCT	Mother-to-child transmission
NAAT	Nucleic acid amplification test
NAT	Nucleic acid test
NCD	National coverage determinations
PALF	Pediatric acute liver failure
PCR	Polymerase chain reaction
POC	Point of care
PrEP	Pre-exposure prophylaxis
PT	Prothrombin time
PWID	People who inject drugs
Rdts	Rapid diagnostic tests
RNA	Ribonucleic acid
SVR	Sustained virologic response
T2DM	Type 2 diabetes mellitus
TB	Tuberculosis
USPSTF	United States Preventive Services Task Force
WHO	World Health Organization

Scientific Background

Hepatitis C

The Centers for Disease Control and Prevention estimate that 2.4 million people in the United States have chronic hepatitis C (CDC, 2020b). Prevalence of the infection is highest in individuals born between 1945 and 1965. This rate is approximately six times higher than that seen in other adult age groups, and the CDC estimated approximately 50,300 new infections occurring each year (CDC, 2018). Hepatitis C virus (HCV) infection is the most common reason for liver transplantation in adults in the U.S. and may lead to hepatocellular carcinoma (Chopra, 2024).

It is estimated that 20% of people with HCV infection will develop cirrhosis, and nearly five percent will die from liver disease resulting from the HCV infection. The number of deaths from hepatitis is increasing and is projected to continue to increase for several more decades unless treatment is scaled up considerably (Razavi et al., 2014). Although HCV infection is common, it is estimated that 50-75% of individuals who are infected are unaware of their infection as symptoms are absent or nonspecific until much later, and therefore do not receive the care and treatment that can mitigate progression to severe liver disease and possibly death (Hagan et al., 2006; Rein et al., 2012).

Hepatitis C virus is spread through exposure to blood of infected individuals. Such exposure includes injection drug use, blood transfusions (prior to 1992), and to a lesser extent, high-risk sexual behaviors. Additionally, being born to an HCV-infected mother, hemodialysis, intranasal drug use, tattoos, incarceration, needle sticks, and invasive procedures (prior to implementation of universal precautions) are also associated with increased risk of HCV infection. Some countries are experiencing a recent resurgence of HCV infection among young intravenous drug users and HIV-infected homosexual men (CDC, 2015; Wandeler et al., 2015).

Hepatitis C virus is a small, positive-stranded RNA-enveloped virus with a highly variable genome (Simmonds, 2001). Assessment of the HCV genotype is crucial for management of the HCV infection. There are currently six major genotypes of HCV, and major treatment decisions (regimen, dosing, duration) vary from genotype to genotype (Chopra & Arora, 2024a). Some regimens for one genotype (such as ledipasvir-sofosbuvir ["Harvoni"] for genotype one) may not be effective for another (in this case, Harvoni may be used for genotypes one, four, five, and six but not two or three) (Lexidrug, 2024; Muir & Graham, 2024).

Hepatitis C virus is frequently asymptomatic, necessitating the need of strong screening procedures. As many as 50% of HCV-infected individuals are unaware of their diagnosis, and risk factors such as drug use or blood transfusions may increase risk of acquiring an HCV infection. Several expert groups, such as the CDC, have delineated screening recommendations in order to provide better care against the virus (Chopra & Arora, 2024b).

Hepatitis C can be diagnosed with either serologic antibody assays or molecular RNA tests. A serologic assay can detect an active infection and a resolved HCV infection, but cannot differentiate whether the infection is acute, chronic, or no longer present. Various serologic assays include enzyme immunoassays (EIA), chemiluminescence immunoassays (CIA), and point-of-care rapid immunoassays (Spach, 2020).

Molecular RNA tests detect Hepatitis C RNA, and the process includes nucleic acid test (NAT) or nucleic acid amplification test (NAAT). The NAT test becomes positive one to two weeks after initial infection and it has become the gold standard test for patients who have a positive EIA screening test. The NAT can detect whether a patient has a current active infection or a resolved infection (Spach, 2020).

Hepatitis B

The hepatitis B virus (HBV) is a double-stranded DNA virus belonging to the hepadnavirus family. The diagnosis of its acute infection is characterized by the detection of hepatitis B surface antigen (HBsAg) and immunoglobulin M (IgM) antibody to hepatitis B core antigen (anti-HBc), and chronic conditions develop in 90% of infants after acute infection at birth, 25%–50% of children newly infected at ages one to five years, and five percent of people newly infected as adults (CDC, 2020a; Lok, 2021).

Hepatitis B virus is transmitted from infected patients to those who are not immune (i.e., hepatitis B surface antibody [anti-HBs]-negative). Methods of transmission include mother-to-child (whether in utero, at birth, or after birth), breastfeeding, paternal transmission (i.e., close contact with infected blood or fluid of fathers), transfusion, sexual transmission, nosocomial infection, percutaneous inoculation, transplantation, and blood exposure via minor breaks in skin or mucous membranes (Teo & Lok, 2022).

In the United States, an estimated 862,000 people were living with chronic hepatitis B infection in 2016, with 21,600 new infections in 2018. Though most people with acute disease recover with no lasting liver damage, 15% to 25% of those with chronic disease develop chronic liver disease, including cirrhosis, liver failure, or liver cancer. It is believed that there are more than 250 million HBV carriers in the world, 600,000 of whom die annually from HBV-related liver diseases. As many as 60% of HBV-infected persons are unaware of their infection, and many remain asymptomatic until the presentation of cirrhosis or late-stage liver disease (CDC, 2020a; Krist et al., 2020; Lok, 2021).

The initial evaluation of chronic HBV infection should include a history and physical examination focusing on "risk factors for coinfection with hepatitis C virus (HCV), hepatitis delta virus (HDV), and/or HIV; use of alcohol; family history of HBV infection and hepatocellular carcinoma (HCC); and signs and symptoms of cirrhosis." Furthermore, it should employ laboratory tests, such as "a complete blood count

with platelets, liver chemistry tests (aspartate aminotransferase [AST], alanine aminotransferase [ALT], total bilirubin, alkaline phosphatase, albumin), international normalized ratio (INR), and tests for HBV replication (HBeAg, antibody to HBeAg [anti-HBe], HBV DNA", and testing for hepatitis A virus (HAV) immunity with HAV immunoglobulin G (IgG) antibody in those who are not immune. Other considerations include evaluation for other causes of liver disease, screening for HIV infection, screening for hepatocellular carcinoma (HCC), screening for fibrosis, and, in rare cases, a liver biopsy (Lok, 2021).

Hepatitis A

Hepatitis A infection is caused by the hepatitis A virus, of which humans are the only known reservoir. The HAV virus is member of the genus *Hepatovirus* in the family Picornaviridae, and other previously used names for HAV infection include epidemic jaundice, acute catarrhal jaundice, and campaign jaundice (Lai & Chopra, 2024).

The hepatitis A virus is generally transmitted through the fecal-oral route, either via person-to-person contact (e.g., transmission within households, within residential institutions, within daycare centers, among military personnel, or sexually) or consumption of contaminated food or water (consumption of undercooked foods or foods infected by food handlers). Additional modes of transmission include blood transfusion and illicit drug use, and it should be noted that maternal-fetal transmission has not yet been described (Lai & Chopra, 2024).

Globally, approximately 1.4 million new cases of HAV infection occur each year—in the United States alone, an estimated 24,900 new infections were detected in 2018. Acute infection by HAV is usually a self-limited disease, with fulminant manifestations of hepatic failure occurring in fewer than one percent of cases. However, symptomatic illness due to HAV still presents itself in seventy percent of adults. Consequently, "diagnosis of acute HAV infection should be suspected in patients with abrupt onset of prodromal symptoms (nausea, anorexia, fever, malaise, or abdominal pain) and jaundice or elevated serum aminotransferase levels, particularly in the setting of known risk factors for hepatitis A transmission" through detection of serum IgM anti-HAV antibodies due to its persistence throughout the duration of the disease (CDC, 2020a; Lai & Chopra, 2024).

Proprietary Testing

Many point-of-care tests have been developed to diagnose hepatitis C efficiently. These point-of-care tests are particularly important for diagnoses in economically impoverished areas. Examples of these tests include OraQuick, TriDot and SDBioline. The OraQuick HCV test is an FDA approved point-of-care test which utilizes a fingerstick and a small whole blood sample to detect the virus. This test is reportedly more than 98% accurate and provides results in 20 minutes (OraSure, 2013). The fourth Generation HCV Tri-Dot is a rapid test which can detect all subtypes of HCV with 100% sensitivity and 98.9% specificity (JMitra&Co, 2015). This test uses human serum or plasma and can provide results in three minutes. Finally, the SDBioline HCV is an immunochromatographic rapid test that can identify HCV antibodies in human serum, plasma, or whole blood (Inc., 2023). This test uses a safe fingerstick procedure to obtain a sample.

Hepatitis panel tests have also been developed. For example, the VIDAS® Hepatitis panel by BioMérieux tests for hepatitis A, B, C, and E in less than two hours. This panel includes 11 automated assays and is a rapid, reliable and simple testing method. (BioMérieux, 2022). Legacy Health's Hepatitis Chronic Panel detects Hepatitis B and C within 24-48 hours through a CIA method (Health, 2023).

A hepatitis C vaccine is currently not available, although many vaccines are under development; barriers to the development of such a vaccine include virus diversity, a lack of knowledge of the immune responses when an infection occurs, and limited models for the testing of new vaccines (Ansaldi et al., 2014; Bailey et al., 2019). The World Health Organization hopes for a 90% reduction in new hepatitis C cases by the year 2030 (Bailey et al., 2019).

Management of HCV infection typically involves monitoring the effect of treatment. The goal of treatment is to achieve a "sustained virologic response" (SVR), which is defined as "an undetectable RNA level 12 weeks following the completion of therapy" (Chopra & Pockros, 2024). This measure is a proxy for elimination of HCV RNA. The assessment schedule may vary regimen to regimen, but the viral load is generally evaluated every few weeks (Chopra & Pockros, 2024).

In 2023, the Biden-Harris administration called on Congress to embrace its proposed five-year program to eliminate hepatitis C in the United States. This five-year program was developed through extensive consultations with key stakeholders from both within and outside of the government, including patient groups, physician groups, and federal agencies. The program aims to significantly expand screening, testing, treatment, prevention, and monitoring of hepatitis C infections in the United States and specifically focuses on populations that are at the greatest risk for infection. One main priority in this national program is to accelerate the availability of point-of-care (POC) diagnostic tests. Hepatitis C RNA diagnostic POC tests are currently available outside of the United States, allowing for a test-and-treat approach in a single visit. "The administration proposal will support the Independent Technology Assessment Program, a collaboration between the Food and Drug Administration and the National Institutes of Health, the speed up clearance or approvals for such tests, just as was done by this same group for COID-19 POC tests." It is believed that the availability of such POC tests will be game-changing for hepatitis C single-visit programs, particularly in "high-impact settings such as community health centers, substance use disorder treatment clinics, correctional facilities, emergency departments, and mobile vans" (Fleurence & Collins, 2023).

Clinical Utility and Validity

In order to determine the link between hepatitis A infection and its rare complication of acute liver failure in children in Somalia, a retrospective study was conducted on children aged 0 to 18 who were admitted to the pediatric outpatient clinic and pediatric emergency departments of the Somalia Mogadishu-Turkey Training and Research Hospital, Somali, from June 2019 and December 2019, and who were tested for HAV and had complete study data available (Keles et al., 2021). The authors found that of the 219 hepatitis A cases analyzed, 25 (11%) were diagnosed with pediatric acute liver failure (PALF) while the remaining 194 were not. It was found that children with PALF had "significantly had more prolonged PT and aPPT, and higher INR values in coagulation assays; and had higher levels of albumin in biochemical tests than the group without liver failure (for all, $p \leq 0.05$)", though no other significant differences were found based on the other laboratory parameters tested. Moreover, "Hepatic encephalopathy was observed in individuals with hepatitis A disease (12/219; 15.4%), in which PALF positive group (5/25;40%) was significantly higher compared to the non-PALF group (7/194; 4%) ($p = < 0{,}001$). The length of stay in the hospital or intensive care unit was significantly higher in children with acute liver failure ($p = 0.001$)". As such, Keles et al. (2021) astutely notes that though "death rates of Hepatitis A infection seem to be low", HAV infection may potentially "require long-term hospitalization of patients due to the complication of acute liver failure, which causes loss of workforce, constitutes a socio-economic burden on individuals and healthcare systems, and leads to mortality in settings where referral pediatric liver transplantation centers are not available".

Spenatto et al. (2013) screened 6194 asymptomatic patients who were requesting an STI screening for hepatitis B infection. The authors found that only "male gender, lack of employment, and birth, in medium or high endemic country, were independently associated with HBsAg positivity in multivariate analysis", and neither sexual behavior nor vaccination status are needed to target high-risk populations (Spenatto et al., 2013).

Su et al. (2022) evaluated the cost-effectiveness of implementing universal HBV screening in China to identify optimal screening strategies. By using a Markov cohort model, the researchers "simulated universal screening scenarios in 15 adult age groups between 18 and 70 years, with different years of screening implementation (2021, 2026, and 2031) and compared to the status quo (i.e., no universal screening)", investigating a total of 180 different scenarios. Their work found suggested that "with a willingness-to-pay level of three times the Chinese gross domestic product (GDP) per capita (US$30 828), all universal screening scenarios in 2021 were cost-effective compared with the status quo", with the "serum HBsAg/HBsAb/HBeAg/HBeAb/HBcAb (five-test) screening strategy in people aged 18-70 years was the most cost-effective strategy in 2021" and "the two-test strategy for people aged 18-70 years became more cost-effective at lower willingness-to-pay levels." Most importantly, they claimed that the "five-test strategy could prevent 3·46 million liver-related deaths in China over the lifetime of the cohort" and that delaying strategic intervention will reduce overall cost-effectiveness (Su et al., 2022).

Messina et al. (2015) performed a meta-analysis on the prevalence of HCV genotypes worldwide. The authors evaluated 1217 studies encompassing approximately 90% of the global population. They calculated genotype one to comprise 83.4 million cases (46.2% of all HCV cases), genotype three to comprise 54.3 million cases (30.1%), and genotypes two, four, and six to comprise a combined 22.8% cases. Genotype five comprised less than one percent of HCV cases. The diversity of genotypes also varied; the highest diversity is observed in China and South-East Asia, while in some countries, such as Egypt and Mongolia, almost all HCV infections are caused by a single genotype (Messina et al., 2015).

Inoue et al. (2017) described four HCV patients whose treatment failed. These four HCV patients had received a treatment regimen of daclatasvir plus asunaprevir, which is used for genotype 1b. However, these four patients were re-tested and found to have a different genotype; three patients had genotype two and the fourth patient had genotype 1a. The authors suggested that the daclatasvir plus asunaprevir regimen was ineffective for patients without genotype 1b (Inoue et al., 2017).

Moreno et al. (2016) performed a cost analysis of expanded HCV coverage. Two scenarios were simulated, one with expanded fibrosis coverage to stage two fibrosis, and the other to all fibrosis cases. Over a 20-year simulation, treatment costs increased, but private payers experienced overall savings of $10 billion to $14 billion after treatment costs. A positive "spillover" benefit of $400 million to Medicare was seen in the five-year model, and a benefit of seven billion dollars to Medicare was seen in the 20-year model (Moreno et al., 2016).

Linthicum et al. (2016) assessed the cost-effectiveness of expanding screening and treatment coverage over a 20-year horizon. The authors investigated three scenarios, each of which expanded coverage to a different stage of fibrosis. "Net social value" was the primary outcome evaluated, and it was calculated by the "value of benefits from improved quality-adjusted survival and reduced transmission minus screening, treatment, and medical costs." Overall, the scenario with only fibrosis stage three and fibrosis stage four covered generated $0.68 billion in social value, but the scenario with all fibrosis patients (stages zero to four) treated produced $824 billion in social value. The authors also noted that the

scenario with all fibrosis stages covered created net social value by year nine whereas the scenario with only stages three and four covered needed all 20 years to break even (Linthicum et al., 2016).

Chen et al. (2019) completed a meta-analysis to research the relationship between type two diabetes mellitus development and patients with a HCV infection. Studies were included from 2010 to 2019. Five types of HCV individuals were incorporated in this study including those who were "non-HCV controls, HCV-cleared patients, chronic HCV patients without cirrhosis, patients with HCV cirrhosis and patients with decompensated HCV cirrhosis" (Chen et al., 2019). HCV infection was found to be a significant risk factor for type two diabetes mellitus development. Further, "HCV clearance spontaneously or through clinical treatment may immediately reduce the risk of the onset and development of T2DM [type 2 diabetes mellitus]" (Chen et al., 2019).

Saeed et al. (2020) completed a systematic review and meta-analysis of health utilities for patients diagnosed with a chronic hepatitis C infection. Health utility can be defined as a measure of health-related quality or general health status. A total of 51 studies comprised of 15,053 patients were included in this study. The researchers have found that "Patients receiving interferon-based treatment had lower utilities than those on interferon-free treatment (0.647 vs 0.733). Patients who achieved sustained virologic response (0.786) had higher utilities than those with mild to moderate CHC [chronic hepatitis C]. Utilities were substantially higher for patients in experimental studies compared to observational studies" (Saeed et al., 2020). Overall, these results show that chronic hepatitis C infections are significantly harming global health status based on the measurements provided by health utility instruments.

Vetter et al. (2022) conducted a retrospective study to assess the performance of rapid diagnostic tests (RDTs) for Hepatitis C virus (HCV) infection. Thirteen RDTs were studied including the Standard Q HCV Ab by SD Biosensor, HCV Hepatitis Virus Antibody Test by Antron Laboratories, HCV-Ab Rapid Test by Beijing Wantal Biological Pharmacy Enterprise, Rapid Anti-HCV Test by InTec, First Response HCV Card Test by Premier Medical Corporation, Signal HCV Version 3.0 by Arkray Healthcare, TRI DOT HCV by J. Mitra & Co, Modified HCV-only Ab Test by Biosynex SA, SD Bioline HCV by Abbott Diagnostics, OraQuick Hepatitis C virus by OraSure, Prototype HCV Ab Test by BioLytical Laboratories, Prototype DPP HCV by Chembio Diagnostic Systems, and Prototype Care Start HCV by Access Bio. A total of 1,710 samples were evaluated in which 648 samples were HCV positive and 264 samples were also HIV positive. In the samples from HIV negative patients, most RDTs showed high sensitivity of > 98% and specificity of >99%. In HIV positive patients, sensitivity was lower with only one RDT reaching >95%. However, specificity was higher, with only four RDTs showing a specificity of <97%. The authors concluded that these tests are compliant with the World Health Organization (WHO) guidance which recommends an HCV RDT to have a sensitivity of >98% and specificity >97%. However, in HIV positive patients, the specificity remained high, but none of the tests met the WHO sensitivity criteria. The authors conclude that "these findings serve as a valuable baseline to investigate RDT performance in prospectively collected whole blood samples in the intended use settings" (Vetter et al., 2022).

In a prospective study, Chevaliez et al. (2020) evaluated the use of molecular point of care (POC) testing and dried blood spot (DBS) for HCV screening in people who inject drugs (PWID). A total of 89 HCV-seropositive PWID were further assessed with a liver assessment, blood tests, POC HCV RNA testing, and fingerstick DBS sampling. A total of 77 patients had paired fingerstick capillary whole blood for POC HCV RNA testing and fingerstick sampling with interpretable results, while the other 12 samples had no valid result due to low sample volume. The POC HCV RNA test detected 30 HCV-seropositive PWID and DBS sampling detected 27 HCV-seropositive PWID. The rate of invalid results using the POC test was below 10%, so it may be performed by staff without extensive clinical training in decentralizing testing

location. This study also showed high concordance for detection of active HCV infection from DBS compared to the POC test. The authors conclude that the use of POC diagnostic testing and DBS sampling should be recommended as a one-step screening strategy to increase diagnosis, increase treatment, and reduce the number of visits.

In an Australian observational study, Catlett et al. (2021) evaluated the Aptima HCV Quant Dx Assay to see how well it could detect HCV RNA from fingerstick capillary dried blood spot (DBS) and venipuncture-collected samples. DBS collection would benefit marginalized populations in areas that may not have access to phlebotomy services or who may have difficult venous access. DBS has also been shown to "enhance HCV testing and linkage to care," be easy for transport and storage, and can be used for other purposes like HCV sequencing and testing for HIV or hepatitis B simultaneously, which is useful in more resource-limited settings. From 164 participants, they found HCV RNA in 45 patients. The Aptima assay rendered a sensitivity and specificity of 100% from plasma, and a sensitivity of 95.6% and specificity of 94.1% from DBS. This demonstrated the comparable diagnostic performance of this assay when it comes to detecting active HCV infection from DBS samples and plasma samples, and hopefully the eventual use of other similar assays with similar performances.

Guidelines and Recommendations

Centers for Disease Control and Prevention (CDC)

Hepatitis C

The CDC recommends universal hepatitis C screening for

- "Hepatitis C screening at least once in a lifetime for all adults aged 18 years and older, except in settings where the prevalence of HCV infection (HCV RNA-positivity) is less than 0.1%"
- "Hepatitis C screening for all pregnant individuals during each pregnancy, except in settings where the prevalence of HCV infection (HCV RNA-positivity) is less than 0.1%" (CDC, 2023a).

Moreover, one-time hepatitis C testing regardless of age or setting prevalence among people with recognized conditions or exposures is recommended for the following groups:

- "People who currently or have previously injected drugs and shared needles, syringes, or other drug preparation equipment.
- People with human immunodeficiency virus (HIV).
- People with selected medical conditions, including
  people who have ever received maintenance hemodialysis and persons with persistently abnormal alanine aminotransferase (ALT) levels.
- Prior recipients of transfusions or organ transplants, including:
  - People who received clotting factor concentrates produced before 1987.
  - People who received a transfusion of blood or blood components before July 1992.
  - People who received an organ transplant before July 1992.
  - People who were notified that they received blood from a donor who later tested positive for HCV infection.
- Health care, emergency medical, and public safety personnel after needle sticks, sharps, or mucosal exposures to HCV-positive blood.
- Infants born to people with known hepatitis C".

It is also stated that "Routine periodic testing is recommended for people with ongoing risk factors (regardless of setting prevalence), including:

- People who currently inject drugs and share needles, syringes, or other drug preparation equipment
- People with selected medical conditions, including

  people who ever received maintenance hemodialysis."

It is also recommended that "Clinicians should test anyone who requests a hepatitis C test, regardless of stated risk factors, because patients may be hesitant to share stigmatizing risks" (CDC, 2023a).

CDC screening and testing guidelines state that "Clinicians should initiate hepatitis C testing with an HCV antibody test with reflex to NAT for HCV RNA if the antibody test is positive/reactive." Moreover, the CDC provides operational guidance for complete hepatitis C testing, noting that "It is important to reduce time to diagnosis, evaluation, and treatment initiation. CDC recommends that clinicians collect all samples needed to diagnose hepatitis C in a single visit and order HCV RNA testing automatically when the HCV antibody is reactive" and that "When the HCV antibody test is reactive, the laboratories should automatically perform NAT testing for HCV RNA detection. This automatic testing streamlines the process because it occurs without any additional action on the part of the patient or the clinician" (CDC, 2023a).

Furthermore, "HCV RNA testing is recommended for the diagnosis of current HCV infection among people who might have been exposed to HCV within the past 6 months, regardless of HCV antibody result."

The CDC asserts that "Clinicians should use an FDA-approved HCV antibody test followed by a NAT for HCV RNA test when antibody is positive/reactive." Such tests include

- HCV antibody test (anti-HCV) (e.g., enzyme immunoassay [EIA]).
- Nucleic acid test (NAT) to detect presence of HCV RNA (qualitative RNA test).
- NAT to detect levels of HCV RNA (quantitative RNA test) (CDC, 2023a).

The CDC notes that "A reactive HCV antibody test result indicates a history of past or current HCV infection. A detectable HCV RNA test result indicates current infection" and urge that "NAT for detection of HCV RNA should be used among people with suspected HCV exposure within the past 6 months."

- For perinatally exposed infants, the CDC notes that "Clinicians should test all perinatally exposed infants for HCV RNA
  - using a NAT at 2–6 months. Care for infants with detectable HCV RNA should be coordinated in consultation with a provider who has expertise in pediatric hepatitis C management.

Infants with undetectable HCV RNA do not require further follow-up unless clinically warranted" (CDC, 2023a).

The CDC also notes that the initial HCV test should be "with an FDA-approved test for antibody to HCV." A positive result for the HCV antibody indicates either a current infection or previous infection that has resolved. For those individuals, the CDC recommends testing by an FDA-approved HCV nucleic acid test (NAT) to differentiate between active infection and resolved infection. For the identification of chronic hepatitis C virus infection among persons born between 1945 and 1965, the CDC states that "Persons

who test anti-HCV positive or have indeterminate antibody test results who are also positive by HCV NAT should be considered to have active HCV infection; these persons need referral for further medical evaluation and care." Finally, the CDC also recommends that repeat testing should be considered for individuals with ongoing risk behaviors (CDC, 2012).

The CDC published guidance for healthcare personnel with potential exposure to HCV. CDC recommends testing the source patient and the healthcare personnel. When testing the source patient, baseline testing should be performed within 48 hours after exposure by testing for HCV RNA or HCV antibodies. All HCV RNA testing should be performed with a nucleic acid test. If the source patient was HCV RNA positive or if source patient testing was not performed, baseline testing for healthcare personnel should follow the same steps through nucleic acid testing three to six weeks post-exposure. A final HCV antibody test should be performed at four to six months post-exposure to ensure a negative HCV RNA test result (CDC, 2020d).

No serologic marker for acute infection is available, but for chronic infections, CDC propounds the use of "Assay for anti-HCV" and "Qualitative and quantitative nucleic acid tests (NAT) to detect and quantify presence of virus (HCV RNA)" (CDC, 2020a).

Hepatitis B

The CDC offers guidance on how to make decisions on whether to test or screen for hepatitis B based on the demographic.

- For adults: "CDC recommends screening all adults aged 18 and older for hepatitis B at least once in their lifetime using a triple panel test

. To ensure increased access to testing, anyone who requests HBV testing should receive it regardless of disclosure of risk. Many people might be reluctant to disclose stigmatizing risks."

For infants: "CDC recommends testing all infants born to HBsAg-positive people for HBsAg and antibody to hepatitis B surface antigen (anti-HBs) seromarkers."

For pregnant people: "CDC recommends HBV screening for HBsAg for all pregnant people during each pregnancy, preferably in the first trimester, regardless of vaccination status or history of testing. Pregnant people with a history of appropriately timed triple panel screening without subsequent risk for exposure to HBV (no new HBV exposures since triple panel screening) only need HBsAg screening."

For people at increased risk: "CDC recommends testing susceptible people periodically, regardless of age, with ongoing risk for exposures while risk for exposures persists. This includes:

- People with a history of sexually transmitted infections or multiple sex partners.
- People with history of past or current HCV infection.
- People incarcerated or formerly incarcerated in a jail, prison, or other detention setting.
- Infants born to HBsAg-positive people.
- People born in regions with HBV infection prevalence of 2% or more.
- US-born people not vaccinated as infants whose parents were born in geographic regions with HBsAg prevalence of 8% or more.
- People who inject drugs or have a history of injection drug use.
- People with human immunodeficiency virus (HIV) infection.
- Men who have sex with men.

- Household contacts or former household contacts of people with known HBV infection.
- People who have shared needles with or engaged in sexual contact with people with known HBV infection.
- People on maintenance dialysis, including in-center or home hemodialysis and peritoneal dialysis.
- People with elevated liver enzymes" (CDC, 2024b).

The CDC also explains that "Susceptible people include those who have never been infected with HBV and either did not complete a HepB vaccine series per ACIP recommendations or who are known to be vaccine nonresponders."

The CDC states that they now* recommend the use of the triple panel test, which includes testing for

- HBsAg
- Anti-HBs
- Total antibody to hepatitis B core antigen (total anti-HBc). This differs from prior guidance (hence the asterisk *), which recommended a single test of HBsAg.

It is noted that "Any periodic follow-up testing can use tests as appropriate based on the results of the triple panel" (CDC, 2024b).

The table below provides CDC recommendations for screening, testing and vaccination for children and adults based on population groups. Infants and Young Adolescents (CDC, 2023c):

	Recommendation	
Population	**Screening and Testing**	**Vaccination**
Infants without known hepatitis B exposure	None	Routine vaccination of all infants with the hepatitis B vaccine series, with the first dose administered within 24 hours of birth See https://www.cdc.gov/hepatitis/hbv/vaccchildren.htm
Infants born to hepatitis B surface antigen (HBsAg)-positive pregnant people	See Perinatal Transmission of Hepatitis B virus | CDC	Provide hepatitis B immune globulin (HBIG) and first dose of hepatitis B vaccine within 12 hours of birth, followed by completion of the vaccine series and postvaccination serologic testing See Hepatitis B Vaccination of Infants – Adolescents | CDC See: Management of Infants Born to Women with Hepatitis B Virus Infection for Pediatricians (cdc.gov)
Infants born to pregnant people for whom HBsAg testing results during pregnancy are not available but for whom other evidence suggests maternal HBV infection	See Perinatal Transmission of Hepatitis B virus | CDC	For infants equal to or more than 2,000 grams, provide first dose of hepatitis B vaccine within 12 hours of birth, followed by completion of the vaccine series

Population	Recommendation	
	Screening and Testing	**Vaccination**
(e.g., HBV DNA, HBeAg-positive, or pregnant person known to be chronically infected with HBV		For infants with birthweight less than 2,000 grams, provide hepatitis B immune globulin (HBIG) and first dose of hepatitis B vaccine within 12 hours of birth, followed by completion of the vaccine series and postvaccination serologic testing See Hepatitis B Vaccination of Infants – Adolescents | CDC
Adolescents under age 19 years who have not been vaccinated and with no known risk factors	None	Vaccinate See Hepatitis B Vaccination of Infants – Adolescents | CDC

Older Adolescents and Adults (CDC, 2023c):

Population	Recommendation	
	Screening and Testing	**Vaccination**
Adults with no known risk factors for hepatitis B	If never previously screened, test for HBsAg, anti-HBs, and total anti-HBc (triple panel)	Vaccinate adults aged 18 – 59 years
People with risk factors, regardless of age, such as: – People born in regions of the world with hepatitis B prevalence >2% – U.S.-born people not vaccinated as infants whose parents were born in regions with hepatitis B prevalence >8% – People with current or past injection drug use – People who share needles, or sexual contacts of people with known HBV infection – People currently or formerly incarcerated in a jail, prison, or other detention setting – People with HIV infection – People with current or past hepatitis C virus infection – who have sex with men – People with current or past sexually transmitted infections, or multiple sex partners	If never previously screened, test for HBsAg, anti-HBs, and total anti-HBc (triple panel) • *Unless* less than aged 18 years and completed a vaccine series as an infant If previously screened, but still unvaccinated, offer testing to people who have ongoing risk for exposure For additional screening considerations for patients on dialysis, see: Recommendations for Preventing Transmission of Infections Among Chronic Hemodialysis Patients (cdc.gov)	Vaccinate For additional considerations for patients on dialysis, see Recommendations for Preventing Transmission of Infections Among Chronic Hemodialysis Patients (cdc.gov)

Population	Recommendation	
	Screening and Testing	**Vaccination**
– Current or former household contacts of people with known HBV infection – People on maintenance dialysis, including in-center or home hemodialysis and peritoneal dialysis, or who are predialysis – People with elevated alanine aminotransferase (ALT) or aspartate aminotransferase (AST) levels of unknown origin		
Other populations at risk: – Residents and staff members of facilities for people with developmental disabilities – Health care and public safety personnel with reasonably anticipated risk for exposure to blood or blood-contaminated body fluids – People with diabetes at the discretion of the treating clinician – International travelers to countries with high or intermediate levels of endemic hepatitis B virus infection	If never previously screened, test for HBsAg, anti-HBs, and total anti-HBc (triple panel) • *Unless* aged <18 years and completed a vaccine series as an infant For additional screening considerations for patients on dialysis see: Recommendations for Preventing Transmission of Infections Among Chronic Hemodialysis Patients (cdc.gov)	Vaccinate For additional vaccination considerations for healthcare personnel see: Prevention of Hepatitis B Virus Infection in the United States: Recommendations of the Advisory Committee on Immunization Practices | MMWR (cdc.gov)

Serologic tests for chronic hepatitis B infections should include three HBV seromarkers: HBsAg, anti-HBs, and Total anti-HBc, while testing for acute infection should include HBsAg and IgM anti-HBc. The CDC provides the following chart on interpreting serologic testing results:

Test outcome	Interpretation	Action
HBsAg — Positive Total anti-HBc — Positive IgM anti-HBc — Positive Anti-HBs — Negative	Acute infection	Link to hepatitis B care
HBsAg — Positive Total anti-HBc — Positive IgM anti-HBc — Negative* Anti-HBs — Negative	Chronic infection	Link to hepatitis B care
HBsAg — Negative Total anti-HBc — Positive Anti-HBs — Positive	Resolved infection	Counsel about HBV infection reactivation risk
HBsAg — Negative Total anti-HBc — Negative Anti-HBs — Positive†	Immune from receipt of prior vaccination (if documented complete series)	If not vaccinated, then complete vaccine series
HBsAg — Negative Total anti-HBc — Positive Anti-HBs — Negative	*Only core antibody is positive. See possible interpretations and corresponding actions.*	
	Resolved infection where anti-HBs levels have waned	Counsel about HBV infection reactivation risk
	Occult infection	Link to hepatitis B care
	Passive transfer of anti-HBc to an infant born to an HBsAg-positive gestational parent	No action
	False positive, thus patient is susceptible	Offer HepB vaccine per ACIP
	A mutant HBsAg strain that is not detectable by laboratory assay	Link to hepatitis B care
HBsAg — Negative Total anti-HBc — Negative Anti-HBs — Negative‡	Susceptible, never infected (if no documentation of HepB vaccine series completion)	Offer HepB vaccine per ACIP recommendations

* IgM anti-HBc also might be positive in persons with chronic infection during severe HBV infection flares or reactivation.

† Immune if anti-HBs concentration is >10 mIU/mL after vaccine series completion.

‡ Anti-HBs concentrations might wane over time among vaccine responders. People with a documented, complete HepB vaccine series typically do not need to be revaccinated, except for special populations like patients on hemodialysis or health care personnel.

Figure 1: Interpreting HBV serologic test results (CDC, 2024b)

For health care providers and viral hepatitis, the CDC makes the following recommendation: "Health care providers should be vaccinated against hepatitis B and tested for hepatitis C after a potential exposure. . . . For continued protection, CDC and the Advisory Committee on Immunization Practices (ACIP) recommend that health-care providers and public-safety workers with reasonably anticipated risk for exposures to blood or infectious body fluids receive the complete hepatitis B vaccine series and have their immunity documented through postvaccination testing" (CDC, 2023b).

Hepatitis A

Hepatitis A does not present as a chronic infection; as such, the CDC offers no testing recommendations (CDC, 2020a). The CDC lists the following clinical features when infected with HAV:

- Abdominal pain, nausea, and/or vomiting
- Dark urine or clay-colored stools
- Diarrhea
- Fatigue
- Fever
- Jaundice
- Joint pain
- Loss of appetite (CDC, 2024a).

However, it should be noted that "In children younger than 6, 70% of infections are asymptomatic. When symptoms do present, young children typically do not have jaundice, whereas most older children and adults with HAV infection have jaundice" (CDC, 2024a).

The CDC cautions that "You will not be able to differentiate hepatitis A virus from other types of viral hepatitis using clinical or epidemiological features alone. Clinicians should conduct test(s) to make an accurate diagnosis." As such, they assert that "The following are laboratory markers that, if present, indicate an acute HAV infection"

- Immunoglobulin M antibodies to HAV (IgM anti-HAV) in serum, or
- HAV RNA in serum or stool (CDC, 2024a).

The CDC notes that the presence of immunoglobulin G antibodies to HAV (IgG anti-HAV) indicates either immunity from prior infection or vaccination.

Not all tests are created equal, however; it should be mentioned that "Serologic tests for IgG anti-HAV and total anti-HAV (IgM and IgG anti-HAV combined) are not helpful in diagnosing acute illness. You should only test patients for IgM anti-HAV if they are symptomatic, and you suspect HAV infection. Alanine aminotransferase (ALT) and total bilirubin tests can aid in diagnosis" (CDC, 2024a).

Hepatitis D

According to the CDC, "HDV is known as a 'satellite virus,' because it can only infect people who are also infected by the hepatitis B virus (HBV). HDV infection can be acute or lead to chronic, long-term illness. The infection can be acquired either simultaneously with HBV as a coinfection or as a superinfection in people who are already chronically infected with HBV" (CDC, 2020c). Hepatitis D infections are not clinically distinguishable from other types of acute viral hepatitis and thus "diagnosis can be confirmed only by testing for the presence of antibodies against HDV and/or HDV RNA. HDV infection should be considered in any person with a positive hepatitis B surface antigen (HBsAg) who has severe symptoms of hepatitis or acute exacerbations" (CDC, 2020c).

United States Preventive Services Task Force (USPSTF)

The USPSTF recommends hepatitis C virus screening in adults aged 18 to 79 years (B recommendation) with anti-HCV antibody testing followed by confirmatory PCR testing (Owens et al., 2020).

The United States Preventive Services Task Force (USPSTF) recommends screening for hepatitis B virus (HBV) infection in adolescents and adults at increased risk for infection. This applies to all asymptomatic,

nonpregnant adolescents and adults at increased risk for HBV infection, including those who were vaccinated before being screened for HBV infection. The USPSTF defines some increased-risk groups as "Persons born in the US with parents from regions with higher prevalence are also at increased risk of HBV infection during birth or early childhood, particularly if they do not receive appropriate passive and active immunoprophylaxis (and antiviral therapy for pregnant individuals with a high viral load)" and also "persons who have injected drugs in the past or currently; men who have sex with men; persons with HIV; and sex partners, needle sharing contacts, and household contacts of persons known to be HBsAg positive" (Krist et al., 2020).

USPSTF recommends the following in relation to screening tests for HBV: "Screening for hepatitis B should be performed with HBsAg tests approved by the US Food and Drug Administration, followed by a confirmatory test for initially reactive results. A positive HBsAg result indicates chronic or acute infection. Serologic panels performed concurrently with or after HBsAg screening allow for diagnosis and to determine further management" (Krist et al., 2020).

American Association for the Study of Liver Diseases (AASLD) and the Infectious Disease Society of America (IDSA)

AASLD-IDSA guidelines recommend one-time HCV testing in the following situations:

- "One-time, routine, opt out HCV testing is recommended for all individuals aged 18 years and older. Rating: I, B
- One-time HCV testing should be performed for all persons less than 18 years old with activities, exposures, or conditions or circumstances associated with an increased risk of HCV infection (see below). Rating: I, B
- Prenatal HCV testing as part of routine prenatal care is recommended with each pregnancy. Rating: I, B
- Periodic repeat HCV testing should be offered to all persons with activities, exposures, or conditions or circumstances associated with an increased risk of HCV exposure (see below). Rating: IIa, C
- Annual HCV testing is recommended for all persons who inject drugs, for HIV-infected men who have unprotected sex with men, and men who have sex with men taking pre-exposure prophylaxis (PrEP). Rating: IIa, C

Risk Activities

  - Injection-drug use (current or ever, including those who injected once)
  - Intranasal illicit drug use
  - Use of glass crack pipes
  - Male engagement in sex with men
  - Engagement in chem sex (defined as the intentional combining of sex with the use of particular nonprescription drugs in order to facilitate or enhance the sexual encounter

Risk exposures

  - Persons on long-term hemodialysis (ever)
  - Persons with percutaneous/parenteral exposures in an unregulated setting
  - Healthcare, emergency medical, and public safety workers after needlestick, sharps, or mucosal exposures to HCV-infected blood
  - Children born to HCV-infected [individuals]

- Recipients of a prior transfusion or organ transplant, including persons who:
  - Were notified that they received blood from a donor who later tested positive for HCV
  - Received a transfusion of blood or blood components, or underwent an organ transplant before July 1992
  - Received clotting factor concentrates produced before 1987.
- Persons who were ever incarcerated

Other considerations and circumstances

- HIV infection
- Sexually active persons about to start pre-exposure prophylaxis (PreP) for HIV
- Chronic liver disease and/or chronic hepatitis, including unexplained elevated alanine aminotransferase (ALT) levels.
- Solid organ donors (living and deceased) and solid organ transplant recipients" (AASLD-IDSA, 2022a)

Recommendations for Initial HCV Testing and Follow-up

- "HCV-antibody testing with reflex HCV RNA polymerase chain reaction (PCR) is recommended for initial HCV testing to establish the presence of active infection (as opposed to spontaneous or treatment-induced viral clearance). Rating: Class I, Level A
- Among persons with a negative HCV-antibody test who were exposed to HCV within the prior six months, HCV-RNA or follow-up HCV-antibody testing six months or longer after exposure is recommended. HCV-RNA testing can also be considered for immunocompromised persons. Rating: Class I, Level C
- Among persons at risk of reinfection after previous spontaneous or treatment-related viral clearance, initial HCV-RNA testing is recommended because a positive HCV-antibody test is expected. Rating: Class I, Level C
- Persons found to have a positive HCV-antibody test and negative results for HCV RNA by PCR should be informed that they do not have evidence of current (active) HCV infection but are not protected from reinfection. Rating: Class I, Level A
- Quantitative HCV-RNA testing is recommended prior to the initiation of antiviral therapy to document the baseline level of viremia (i.e., baseline viral load).
  Rating: Class I, Level A
- HCV genotype testing may be considered for those in whom it may alter treatment recommendations. Rating: Class I, Level A" (AASLD-IDSA, 2022a; Bhattacharya et al., 2023)

For diagnosing and monitoring acute HCV infection, AASLD-IDSA issued the following recommendation:

- "HCV antibody and HCV RNA testing are recommended when acute HCV infection is suspected due to exposure, clinical presentation, or elevated aminotransferase levels." (Rating: Class I, Level C)
- "After the initial diagnosis of acute HCV with viremia (defined as quantifiable RNA), HCV treatment should be initiated without awaiting spontaneous resolution."(Rating: Class I, Level B) (AASLD-IDSA, 2022b)

For monitoring patients who are starting hepatitis C treatment, are on treatment, or have completed therapy, AASLD-IDSA issued the following recommendations:

- "The following laboratory tests are recommended within six months prior to starting DAA (direct-acting antiviral) therapy:
  - Complete blood count (CBC)
  - International normalized ratio (INR)
  - Hepatic function panel (i.e., serum albumin, total and direct bilirubin, alanine aminotransferase [ALT], aspartate aminotransferase [AST], and alkaline phosphatase levels)
  - Estimated glomerular filtration rate (eGFR)
- The following laboratory tests are recommended any time prior to starting DAA therapy:
  - Quantitative HCV RNA (HCV viral load)
  - If a nonpangenotypic DAA will be prescribed, then test for HCV genotype and subtype" (Rating: Class I, Level C)
- "Quantitative HCV viral load testing is recommended 12 or more weeks after completion of therapy to document sustained virologic response (SVR), which is consistent with cure of chronic infection" (Rating: Class I, Level B) (AASLD-IDSA, 2023b)

Recommendations for Posttreatment Follow-Up for Patients in Whom Treatment Failed

- "Disease progression assessment every six to 12 months with a hepatic function panel, complete blood count (CBC), and international normalized ratio (INR) is recommended if patients are not retreated or fail a second or third DAA treatment course. (Rating: Class I, Level C)
- Surveillance for hepatocellular carcinoma with liver ultrasound examination, with or without alpha fetoprotein (AFP), every six months is recommended for patients with cirrhosis in accordance with the AASLD guidance on the diagnosis, staging, and management of hepatocellular carcinoma. Rating: Low, Conditional" (AASLD-IDSA, 2023b).

Recommendations for Monitoring HCV-Infected [Persons] During Pregnancy

- "As part of prenatal care, all pregnant [individuals] should be tested for HCV infection with each pregnancy, ideally at the initial visit. (Rating: I, B)
- HCV RNA and routine liver function tests are recommended at initiation of prenatal care for HCV-antibody–positive pregnant persons to assess the risk of mother-to-child transmission (MTCT) and severity of liver disease. (Rating: I, B)
- All pregnant individuals with HCV infection should receive prenatal and intrapartum care that is appropriate for their individual obstetric risk(s) as there is no currently known intervention to reduce MTCT. (Rating: I, B)
- In HCV-infected pregnant individuals with pruritus or jaundice, there should be a high index of suspicion for intrahepatic cholestasis of pregnancy (ICP) with subsequent assessment of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and serum bile acids. (Rating: I, B)
- HCV-infected individuals with cirrhosis should be counseled about the increased risk of adverse maternal and perinatal outcomes. Antenatal and perinatal care should be coordinated with a maternal-fetal medicine (i.e., high-risk pregnancy) obstetrician (Rating: I, B)" (AASLD-IDSA, 2023a).

Assessment of Liver Disease Severity

A section focused on determining the severity of liver diseases associated with an HCV infection is also included as part of the background of these AASLD-IDSA guidelines. The authors state the following:

"The severity of liver disease associated with chronic HCV infection is a key factor in determining the initial and follow-up evaluation of patients. Noninvasive tests using serum biomarkers, elastography, or

liver imaging allow for accurate diagnosis of cirrhosis in most individuals (see pretreatment workup in When and in Whom to Initiate HCV Therapy). Liver biopsy is rarely required but may be considered if other causes of liver disease are suspected.

Noninvasive methods frequently used to estimate liver disease severity include:

- Liver-directed physical exam (normal in most patients)
- Routine blood tests (eg, ALT, AST, albumin, bilirubin, international normalized ratio [INR], and CBC with platelet count)
- Serum fibrosis marker panels
- Transient elastography
- Liver imaging (eg, ultrasound or CT scan)" (AASLD-IDSA, 2022a)

Testing of Perinatally Exposed Children and Siblings of Children with HCV Infection

- All children born to individuals with acute or chronic hepatitis C should be tested for HCV infection.
- Antibody-based testing is recommended at or after 18 months of age. (I, A)
- Testing with an HCV-RNA assay can be considered in the first year of life, but the optimal timing of such testing is unknown. (IIa, C)
- Testing with an HCV-RNA assay can be considered as early as two months of age. (IIa, B)
- Repetitive HCV-RNA testing prior to 18 months of age is not recommended. (III, A)
- Children who are anti-HCV-positive after 18 months of age should be tested with an HCV RNA assay after age three to confirm chronic hepatitis C infection. (I, A)
- The siblings of children with vertically acquired chronic hepatitis C should be tested for HCV infection, if born from the same mother (I, C) (Ghany & Morgan, 2020)

Testing recommendations relating to the monitoring and medical management of children include

- "Routine liver biochemistries at initial diagnosis and at least annually thereafter are recommended to assess for HCV disease progression. (I, C)"
- "Disease severity assessment by routine laboratory testing and physical examination, as well as use of evolving noninvasive modalities (i.e., transient elastography, imaging, or serum fibrosis markers) is recommended for all children with chronic hepatitis C. (I, B)" (Ghany & Morgan, 2020).

American Association for the Study of Liver Diseases (AASLD)

Hepatitis B

The guidance statements surrounding screening for hepatitis B infection is (shown in more detail following declare that

1. Screening should be performed using both HBsAg and anti-HBs.
2. Screening is recommended in all persons born in countries with a HBsAg seroprevalence of ≥2%, U.S.-born persons not vaccinated as infants whose parents were born in regions with high HBV endemicity (≥8%), pregnant individuals, persons needing immunosuppressive therapy, and the at-risk groups listed in Table 3.
3. Anti-HBs–negative screened persons should be vaccinated.

4. Screening for anti-HBc to determine prior expo-sure is not routinely recommended but is an important test in patients who have HIV infection, who are about to undergo HCV or anti-cancer and other immunosuppressive therapies or renal dialysis, and in donated blood (or, if feasible, organs) (Terrault et al., 2018).

AASLD recommends that the following groups are at high risk for HBV infection and should be screened and immunized if seronegative (Terrault et al., 2018):

TABLE 3. Groups at High Risk for HBV Infection Who Should Be Screened

- Persons born in regions of high or intermediate HBV endemicity (HBsAg prevalence of ≥2%)
  - Africa (all countries)
  - North, Southeast, East Asia (all countries)
  - Australia and South Pacific (all countries except Australia and New Zealand)
  - Middle East (all countries except Cyprus and Israel)
  - Eastern Europe (all countries except Hungary)
  - Western Europe (Malta, Spain, and indigenous populations of Greenland)
  - North America (Alaskan natives and indigenous populations of Northern Canada)
  - Mexico and Central America (Guatemala and Honduras)
  - South America (Ecuador, Guyana, Suriname, Venezuela, and Amazonian areas)
  - Caribbean (Antigua-Barbuda, Dominica, Grenada, Haiti, Jamaica, Saint Kitts and Nevis, Saint Lucia, and Turks and Caicos Islands)
- U.S.-born persons not vaccinated as an infant whose parents were born in regions with high HBV endemicity (≥8%)*
- Persons who have ever injected drugs*
- Men who have sex with men*
- Persons needing immunosuppressive therapy, including chemotherapy, immunosuppression related to organ transplantation, and immunosuppression for rheumatological or gastroenterologic disorders.
- Individuals with elevated ALT or AST of unknown etiology*
- Donors of blood, plasma, organs, tissues, or semen
- Persons with end-stage renal disease, including predialysis, hemodialysis, peritoneal dialysis, and home dialysis patients*
- All pregnant women
- Infants born to HBsAg-positive mothers*
- Persons with chronic liver disease, e.g., HCV*
- Persons with HIV*
- Household, needle-sharing, and sexual contacts of HBsAg-positive persons*
- Persons who are not in a long-term, mutually monogamous relationship (e.g., >1 sex partner during the previous 6 months)*
- Persons seeking evaluation or treatment for a sexually transmitted disease*
- Health care and public safety workers at risk for occupational exposure to blood or blood-contaminated body fluids*
- Residents and staff of facilities for developmentally disabled persons*
- Travelers to countries with intermediate or high prevalence of HBV infection*
- Persons who are the source of blood or body fluid exposures that might require postexposure prophylaxis
- Inmates of correctional facilities*
- Unvaccinated persons with diabetes who are aged 19 through 59 years (discretion of clinician for unvaccinated adults with diabetes who are aged ≥60 years)*

*Indicates those who should receive hepatitis B vaccine, if seronegative.
Sources: [23,35,36].

AASLD proposes the use of various screening methods for the diagnose of hepatitis B infection: "HBsAg and antibody to hepatitis B surface antigen (anti-HBs) should be used for screening (Table 4). Alternatively, antibody to hepatitis B core antigen (anti-HBc) can be utilized for screening as long as those who test positive are further tested for both HBsAg and anti-HBs to differentiate current infection from previous HBV exposure. HBV vaccination does not lead to anti-HBc positivity." The interpretations and follow-up steps of the screening results are summarized in their table (Terrault et al., 2018):

TABLE 4. Interpretation of Screening Tests for HBV Infection

Screening Test Results					
HBsAg	Anti-HBc	Anti-HBs	Interpretation	Management	Vaccinate?
+	+	–	Chronic hepatitis B	Additional testing and management needed	No
–	+	+	Past HBV infection, resolved	No further management unless immunocompromised or undergoing chemotherapy or immunosuppressive therapy	No
–	+	–	Past HBV infection, resolved or false-positive	HBV DNA testing if immunocompromised patient	Yes, if not from area of intermediate or high endemicity
–	–	+	Immune	No further testing	No
–	–	–	Uninfected and not immune	No further testing	Yes

Hepatitis C

AASLD recommends not repeating hepatitis C viral load testing in patients with a previous positive (HCV) test, stating that "repeat HCV antibody testing adds cost but no clinical benefit." They recommend "Instead, order hepatitis C viral load testing for assessment of active versus resolved infection." This recommendation is also sponsored by the American Society for Clinical Pathology (AASLD, 2023).

World Health Organization (WHO)

Hepatitis C

Recommendations on screening for HCV infection (WHO, 2017, 2018):

Testing approach	Recommendations
Focused testing in most affected populations	In all settings (and regardless of whether delivered through facility- or community-based testing), it is recommended that serological testing for HCV antibody (anti-HCV) be offered with linkage to prevention, care and treatment services to the following: • Adults and adolescents from populations most affected by HCV infection (i.e. who are either part of a population with high HCV seroprevalence or who have a history of exposure and/or high-risk behaviors for HCV infection); • Adults, adolescents and children with a clinical suspicion of chronic viral hepatitis (i.e. symptoms, signs, laboratory markers) (strong recommendation, low quality of evidence) *Note: Periodic re-testing using HCV NAT should be considered for those with ongoing risk of acquisition or reinfection.*
General population testing	In settings with a ≥2% (intermediate) or ≥5% (high) HCV antibody seroprevalence in the general population, it is recommended that all adults have access to and be offered HCV serological testing with linkage to prevention, care and treatment services. General population testing approaches should make use of existing community- or facility-based testing opportunities or programs such as HIV or TB clinics, drug treatment services and antenatal clinics (conditional recommendation, low quality of evidence

Which serological assay to use	To test for serological evidence of past or present infection in adults, adolescents and children (>18 months of age), an HCV serological assay (antibody or antibody/antigen) using either a rapid diagnostic test (RDT) or laboratory-based immunoassay formats that meet minimum safety, quality and performance standards (with regard to both analytical and clinical sensitivity and specificity) is recommended. • In settings where there is limited access to laboratory infrastructure and testing, and/or in populations where access to rapid testing would facilitate linkage to care and treatment, RDTs are recommended. (Strong recommendation, low/moderate quality of evidence)

In a guideline pertaining to the screening, care, and treatment of people with chronic hepatitis C infection, the WHO has provided the following recommendations on hepatitis C screening:

- "Who to test for HCV infection?
    1. **Focused testing in most-affected populations.** In all settings (and regardless of whether delivered through facility- or community-based testing), it is recommended that serological testing for HCV antibody (anti-HCV)1 be offered with linkage to prevention, care and treatment services to the following individuals:
        - Adults and adolescents from populations most affected by HCV infection (i.e. who are either part of a population with high HCV seroprevalence or who have a history of exposure and/or high-risk behaviours for HCV infection);
        - Adults, adolescents and children with a clinical suspicion of chronic viral hepatitis (i.e. symptoms, signs, laboratory markers). (Strong recommendation, low quality of evidence)
        - Note: Periodic retesting using HCV nucleic acid tests (NAT) should be considered for those with ongoing risk of acquisition or reinfection.
    2. **General population testing.** In settings with a ≥2% or ≥5%4 HCV antibody seroprevalence in the general population, it is recommended that all adults have access to and be offered HCV serological testing with linkage to prevention, care and treatment services.
        - General population testing approaches should make use of existing community- or facility based testing opportunities or programmes such as HIV or TB clinics, drug treatment services and antenatal clinics. (Conditional recommendation, low quality of evidence)
    3. **Birth cohort testing.** This approach may be applied to specific identified birth cohorts of older persons at higher risk of infection and morbidity within populations that have an overall lower general prevalence. (Conditional recommendation, low quality of evidence)"
- "How to test for chronic HCV infection and monitor treatment response?
    1. **Which serological assay to use?** To test for serological evidence of past or present infection in adults, adolescents and children (>18 months of age), an HCV serological assay (antibody or antibody/antigen) using either a rapid diagnostic test (RDT) or laboratory-based immunoassay formats that meet minimum safety, quality and performance standards (with regard to both analytical and clinical sensitivity and specificity) is recommended.
        - In settings where there is limited access to laboratory infrastructure and testing, and/or in populations where access to rapid testing would facilitate linkage to care and treatment, RDTs are recommended. (Strong recommendation, low/moderate quality of evidence)
    2. **Serological testing strategies.** In adults and children older than 18 months, a single serological assay for initial detection of serological evidence of past or present infection is

recommended prior to supplementary nucleic acid testing (NAT) for evidence of viraemic infection. (Conditional recommendation, low quality of evidence)

3. **Detection of viraemic infection**
    - Directly following a reactive HCV antibody serological test result, the use of quantitative or qualitative NAT for detection of HCV RNA is recommended as the preferred strategy to diagnose viraemic infection. (Strong recommendation, moderate/low quality of evidence)
    - An assay to detect HCV core (p22) antigen, which has comparable clinical sensitivity to NAT, is an alternative to NAT to diagnose viraemic infection. (Conditional recommendation, moderate quality of evidence)
4. **Assessment of HCV treatment response**
    - Nucleic acid testing for qualitative or quantitative detection of HCV RNA should be used as the test of cure at 12 or 24 weeks (i.e. sustained virological response [SVR12 or SVR24]) after completion of antiviral treatment. (Conditional recommendation, moderate/low quality of evidence)" (WHO, 2018).

The WHO also includes a table which shows the populations with a high HCV prevalence or who have a history of HCV risk. The following groups are included:

- "Persons who have received medical or dental interventions in health-care settings where infection control practices are substandard
- Persons who have received blood transfusions prior to the time when serological testing of blood donors for HCV was initiated or in countries where serological testing of blood donations for HCV is not routinely performed
- People who inject drugs (PWID)
- Persons who have had tattoos, body piercing or scarification procedures done where infection control practices are substandard
- Children born to mothers infected with HCV
- Persons with HIV infection
- Persons who use/have used intranasal drugs
- Prisoners and previously incarcerated persons" (WHO, 2016)

The WHO also mentions liver function tests several times, stating that "A number of clinical considerations are important for the management of persons with chronic HCV infection"; further, "Pre-treatment evaluation of the risk of adverse events should be based on the patient's clinical details, concomitant medications, and knowledge of treatment regimen to be administered. The potential for DDIs [drug-drug interactions] should be assessed before treatment, and a regimen that has a low risk of DDI selected. Standard laboratory tests that are assessed prior to treatment initiation include a full blood count (FBC), international normalized ratio (INR), renal function and liver function tests: ALT, AST, bilirubin, albumin and alkaline phosphatase" (WHO, 2016).

The WHO also mentions that "in persons with HCV infection being treated for TB, it is important to monitor liver function tests" and that "Baseline liver function tests for individuals with chronic liver disease are encouraged prior to initiating treatment for latent TB infection. For individuals with abnormal baseline test results, routine periodic laboratory testing should be carried out during the treatment of latent TB infection" (WHO, 2016).

The recommendations of the WHO for assays and strategies regarding hepatitis C testing are summarized in their table, captured below (WHO, 2017):

HOW TO TEST FOR CHRONIC HCV INFECTION AND MONITOR TREATMENT RESPONSE	
Topic	**Recommendations***
Which serological assays to use	• To test for serological evidence of past or present infection in adults, adolescents and children (>18 months of age[1]), an HCV serological assay (antibody or antibody/antigen) using either RDT or laboratory-based immunoassay formats[2] that meet minimum safety, quality and performance standards[3] (*with regard to both analytical and clinical sensitivity and specificity*) is recommended. - In settings where there is limited access to laboratory infrastructure and testing, and/or in populations where access to rapid testing would facilitate linkage to care and treatment, RDTs are recommended. *Strong recommendation, low/moderate quality of evidence*
Serological testing strategies	In adults and children older than 18 months[1], a single serological assay for initial detection of serological evidence of past or present infection is recommended prior to supplementary nucleic acid testing (NAT) for evidence of viraemic infection. *Conditional recommendation, low quality of evidence*
Detection of viraemic infection	• Directly following a reactive HCV antibody serological test result, the use of quantitative or qualitative NAT for detection of HCV RNA is recommended as the preferred strategy to diagnose viraemic infection. *Strong recommendation, moderate/low quality of evidence* • An assay to detect HCV core (p22) antigen, which has comparable clinical sensitivity to NAT, is an alternative to NAT to diagnose viraemic infection[4]. *Conditional recommendation, moderate quality of evidence*
Assessment of HCV treatment response	• Nucleic acid testing for qualitative or quantitative detection of HCV RNA should be used as test of cure at 12 or 24 weeks (i.e. sustained virological response (SVR12 or SVR24)) after completion of antiviral treatment. *Conditional recommendation, moderate/low quality of evidence*

Hepatitis B

The below table details the populations who should be tested for chronic hepatitis B infection, according to the WHO (WHO, 2017):

WHO TO TEST FOR CHRONIC HBV INFECTION	
Testing approach and population	**Recommendations***
General population testing	1. In settings with a ≥2% or ≥5%[1] HBsAg seroprevalence in the general population, it is recommended that all adults have routine access to and be offered HBsAg serological testing with linkage to prevention, care and treatment services. General population testing approaches should make use of existing community- or health facility-based testing opportunities or programmes such as at antenatal clinics, HIV or TB clinics. *Conditional recommendation, low quality of evidence*
Routine testing in pregnant women	2. In settings with a ≥2% or ≥5%%[1] HBsAg seroprevalence in the general population, it is recommended that HBsAg serological testing be routinely offered to all pregnant women in antenatal clinics[2], with linkage to prevention, care and treatment services. Couples and partners in antenatal care settings should be offered HBV testing services. *Strong recommendation, low quality of evidence*
Focused testing in most affected populations	*3.* In all settings (and regardless of whether delivered through facility- or community-based testing), it is recommended that HBsAg serological testing and linkage to care and treatment services be offered to the following individuals: • **Adults and adolescents from populations most affected by HBV infection[3]** (i.e. who are either part of a population with high HBV seroprevalence or who have a history of exposure and/or high-risk behaviours for HBV infection); • **Adults, adolescents and children with a clinical suspicion of chronic viral hepatitis[4]** (i.e. symptoms, signs, laboratory markers); • **Sexual partners, children and other family members, and close household contacts** of those with HBV infection[5]; • **Health-care workers:** in all settings, it is recommended that HBsAg serological testing be offered and hepatitis B vaccination given to all health-care workers who have not been vaccinated previously (*adapted from existing guidance on hepatitis B vaccination[6]*) *Strong recommendation, low quality of evidence*
Blood donors *Adapted from existing 2010 WHO guidance (Screening donated blood for transfusion transmissible infections[7])*	4. In all settings, screening of blood donors should be mandatory with linkage to care, counselling and treatment for those who test positive.

Similarly, the recommendations of the WHO for assays and strategies regarding hepatitis B testing are summarized in their table, captured below (WHO, 2017):

HOW TO TEST FOR CHRONIC HBV INFECTION AND MONITOR TREATMENT RESPONSE	
Topic	**Recommendations***
Which serological assays to use	• For the diagnosis of chronic HBV infection in adults, adolescents and children (>12 months of age[1]), a serological assay (in either RDT or laboratory-based immunoassay format[2]) that meets minimum quality, safety and performance standards[3] (*with regard to both analytical and clinical sensitivity and specificity*) is recommended to detect hepatitis B surface antigen (HBsAg). - In settings where existing laboratory testing is already available and accessible, laboratory-based immunoassays are recommended as the preferred assay format. - In settings where there is limited access to laboratory testing and/or in populations where access to rapid testing would facilitate linkage to care and treatment, use of RDTs is recommended to improve access. *Strong recommendation, low/moderate quality of evidence*
Serological testing strategies	• In settings or populations with an HBsAg seroprevalence of ≥0.4%[4], a single serological assay for detection of HBsAg is recommended, prior to further evaluation for HBV DNA and staging of liver disease. • In settings or populations with a low HBsAg seroprevalence of <0.4%[4], confirmation of HBsAg positivity on the same immunoassay with a neutralization step or a second different RDT assay for detection of HBsAg may be considered[5]. *Conditional recommendation, low quality of evidence*
Detection of HBV DNA – assessment for treatment *Adapted from existing guidance (WHO HBV 2015 guidelines[6])*	• Directly following a positive HBsAg serological test, the use of quantitative or qualitative nucleic acid testing (NAT) for detection of HBV DNA is recommended as the preferred strategy and to guide who to treat or not treat. *Strong recommendation, moderate/low quality of evidence*
Monitoring for HBV treatment response and disease progression *Existing guidance (WHO HBV 2015 guidelines[6])*	• **It is recommended that the following be monitored at least annually:** - ALT levels (and AST for APRI), HBsAg[7], HBeAg[8], and HBV DNA levels (where HBV DNA testing is available) - Non-invasive tests (APRI score or transient elastography) to assess for presence of cirrhosis in those without cirrhosis at baseline; - If on treatment, adherence should be monitored regularly and at each visit. *Strong recommendation, moderate quality of evidence* **More frequent monitoring is recommended:** • **In persons on treatment or following treatment discontinuation:** more frequent on-treatment monitoring (at least every 3 months for the first year) is indicated in: persons with more advanced disease (compensated or decompensated cirrhosis[9]); during the first year of treatment to assess treatment response and adherence; where treatment adherence is a concern; in HIV-coinfected persons; and in persons after discontinuation of treatment. *Conditional recommendation, very low quality of evidence* • **In persons who do not yet meet the criteria for antiviral therapy:** i.e. persons who have intermittently abnormal ALT levels or HBV DNA levels that fluctuate between 2000 IU/mL and 20 000 IU/mL (where HBV DNA testing is available) and in HIV-coinfected persons[7]. *Conditional recommendation, low quality of evidence*

Hepatitis D

For the diagnosis of hepatitis D, the WHO states that HDV infection is "diagnosed by high levels of anti-HDV immunoglobulin G (IgG) and immunoglobulin M (IgM), and confirmed by detection of HDV RNA in serum. However, HDV diagnostics are not widely available and there is no standardization for HDV RNA assays, which are used for monitoring response to antiviral therapy"(WHO, 2023).

American Gastroenterological Association (AGA)

Hepatitis B

"The AGA recommends screening for HBV (HBsAg and anti-HBc, followed by a sensitive HBV DNA test if positive) in patients at moderate or high risk who will undergo immunosuppressive drug therapy. (*Strong recommendation; Moderate-quality evidence*) The AGA suggests against routinely screening for HBV in patients who will undergo immunosuppressive drug therapy and are at low risk. (*Weak recommendation; Moderate-quality evidence*) Comments: *Patients in populations with a baseline*

prevalence likely exceeding 2% for chronic HBV should be screened according to Centers for Disease Control and Prevention and US Preventive Services Task Force recommendations" (Reddy et al., 2015).

Hepatitis C

The AGA released best practice statements for care of patients with chronic HCV that have achieved a sustained virologic response (SVR).

- "SVR should be confirmed by undetectable HCV RNA at 12 weeks after completion of an all-oral DAA treatment regimen."
- "Routine confirmation of SVR at 48 weeks post end of treatment is recommended. Testing for HCV RNA at 24 weeks post treatment should be considered on an individual patient basis."
- "Routine testing for HCV RNA beyond 48 weeks after end of treatment to evaluate for late virologic relapse is not supported by available evidence; periodic testing for HCV RNA is recommended for patients with ongoing risk factors for reinfection" (Jacobson et al., 2017).

The AGA has also released a "pathway" for HCV treatment (an algorithm).

Prior to treatment, the AGA recommends identifying the HCV genotype, as well as taking a hepatic function panel (defined as albumin, total and direct bilirubin, alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase).

For all three lengths of treatment courses (8, 12, 16 weeks), the AGA recommends assessing viral load and liver function (the same hepatic panel listed above) (Kanwal et al., 2017).

European Association for the Study of the Liver (EASL)

Hepatitis C

The EASL released guidelines on treatment of hepatitis C. The EASL recommends:

- "Screening strategies for HCV infection should be defined according to the local epidemiology of HCV infection, ideally within the framework of local, regional or national action plans.
- Liver disease severity must be assessed prior to therapy.
- Rapid diagnostic tests using serum, plasma, fingerstick whole blood or crevicular fluid (saliva) as matrices can be used instead of classical EIAs as point-of-care tests to facilitate anti-HCV antibody screening and improve access to care.
- "It is still useful to determine the HCV genotype and subtype where such determination is available and does not limit access to care, to identify patients who may benefit from treatment tailoring. However, "testing for HCV resistance prior to treatment is not recommended" (EASL, 2020).

Hepatitis B

The EASL states that "The initial evaluation of a subject with chronic HBV infection should include a complete history, a physical examination, assessment of liver disease activity and severity and markers of HBV infection (Fig. 1). In addition, all first-degree relatives and sexual partners of subjects with chronic HBV infection should be advised to be tested for HBV serological markers (HBsAg, anti-HBs, anti-HBc) and to be vaccinated if they are negative for these markers."

"The assessment of the severity of liver disease is important to identify patients for treatment and HCC surveillance. It is based on a physical examination and biochemical parameters (aspartate aminotransferase [AST] and ALT, gamma-glutamyl transpeptidase [GGT], alkaline phosphatase, bilirubin, and serum albumin and gamma globulins, full blood count and prothrombin time). An abdominal hepatic ultrasound is recommended in all patients. A liver biopsy or a non-invasive test should be performed to determine disease activity in cases where biochemical and HBV markers reveal inconclusive results."

"HBeAg and anti-HBe detection are essential for the determination of the phase of chronic HBV infection."

"Measurement of HBV DNA serum level is essential for the diagnosis, establishment of the phase of the infection, the decision to treat and subsequent monitoring of patients."

"Serum HBsAg quantification can be useful, particularly in HBeAg-negative chronic HBV infection and in patients to be treated with interferon-alfa (IFNα)."

"HBV genotype is not necessary in the initial evaluation, although it may be useful for selecting patients to be treated with IFNα offering prognostic information for the probability of response to IFNα therapy and the risk of HCC."

"Co-morbidities, including alcoholic, autoimmune, metabolic liver disease with steatosis or steatohepatitis and other causes of chronic liver disease should be systematically excluded including co-infections with hepatitis D virus (HDV), hepatitis C virus (HCV) and HIV."

"Testing for antibodies against hepatitis A virus (anti-HAV) should be performed, and patients with negative anti-HAV should be advised to be vaccinated against HAV."

"Screening for HBsAg in the first trimester of pregnancy is strongly recommended (Evidence level 1, grade of recommendation 1)" (Lampertico et al., 2017).

Hepatitis D

In the clinical practice guidelines for Hepatitis D, the EASL provided the following recommendations:

- "Screening for anti-HDV antibodies should be performed with a validated assay at least once in all HBsAg-positive individuals (LoE 3, strong recommendation, strong consensus).
- Re-testing for anti-HDV antibodies should be performed in HBsAg-positive individuals whenever clinically indicated (e.g., in case of aminotransferase flares, or acute decompensation of chronic liver disease) (LoE 3, strong recommendation, strong consensus), and may be performed yearly in those remaining at risk of infection (LoE 5, weak recommendation, strong consensus)
- HDV RNA should be tested in all anti-HDV-positive individuals using a standardised and sensitive reverse transcription PCR assay to diagnose active HDV infection (LoE 2, strong recommendation, strong consensus).
- In patients with acute hepatitis, anti-HBc IgM should be used to distinguish individuals with HBV/HDV coinfection from HBsAg-positive individuals superinfected with HDV (LoE 3; strong recommendation, consensus).

- HBV e antigen (HBeAg)/anti-HBe status and HBV DNA levels should be tested because the presence of active HBV infection may worsen the outcome of hepatitis D (LoE 3; strong recommendation, consensus)" (Brunetto et al., 2023).

In this clinical guideline, the EASL notes the following concerning quantitative RNA monitoring for HDV: "Preliminary reports suggest that viral load correlates with disease activity and progression; however, further studies with standardised assays are required to confirm these findings and define the prognostic role of quantitative HDV RNA monitoring in untreated patients" (Brunetto et al., 2023).

Indian Health Services (IHS)

Indian Health Services published recommendations on Hepatitis C screening. IHS recommends using an anti-HCV antibody test such as a point-of-care test on a fingerstick capillary or venipuncture whole-blood sample or a laboratory-based HCV ELISA test on a serum sample. IHS recommends screening the following patients:

- "Adults 18 years and older, including people with diabetes, at least once for HCV infection, regardless of their risk factors.
- All pregnant persons, regardless of age, during each pregnancy.
- People at higher risk of HCV exposure" (IHS, 2021).

IHS also provides guidance on how to diagnose a chronic HCV infection:

- "For individuals with a positive HCV antibody screening test result, perform the laboratory-based HCV RNA PCR test to confirm the presence of HCV.
    - The presence of HCV indicates active infection. These individuals should be referred for direct acting anti-viral (DAA) agents treatment.
    - The absence of HCV indicates no active infection.
- For individuals with a negative HCV antibody test result who might have been exposed to HCV within the previous six months, perform an HCV RNA PCR or follow-up HCV antibody test at least six months after exposure" (IHS, 2021).

Regarding hepatitis B, the IHS suggests that

"People who inject drugs illicitly, including participants in substance abuse treatment programs, should be offered screening and counseling for chronic HBV infection. Testing should include a serologic assay for hepatitis B surface antigen (HBsAg) offered as a part of routine care, and if the result is positive, be accompanied by appropriate counseling and referral for recommended clinical evaluation and care. Previous and current sex partners and household and needle-sharing contacts of HBsAg-positive persons should be identified. Unvaccinated sex partners and household and needle-sharing contacts should be tested for HBsAg and for antibody to the hepatitis B core antigen (anti-HBc) or antibody to the hepatitis B surface antigen (anti-HBsAg)" (IHS, 2022).

Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the

Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
86692	Antibody; hepatitis, delta agent
86704	Hepatitis B core antibody (HBcAb); total
86705	Hepatitis B core antibody (HBcAb); IgM antibody
86706	Hepatitis B surface antibody (HBsAb)
86708	Hepatitis A antibody (HAAb)
86709	Hepatitis A antibody (HAAb), IgM antibody
86803	Hepatitis C antibody
86804	Hepatitis C antibody; confirmatory test (eg, immunoblot)
87340	Infectious agent antigen detection by immunoassay technique, (eg, enzyme immunoassay [EIA], enzyme-linked immunosorbent assay [ELISA], fluorescence immunoassay [FIA], immunochemiluminometric assay [IMCA]) qualitative or semiquantitative; hepatitis B surface antigen (HBsAg)
87341	Infectious agent antigen detection by immunoassay technique, (eg, enzyme immunoassay [EIA], enzyme-linked immunosorbent assay [ELISA], fluorescence immunoassay [FIA], immunochemiluminometric assay [IMCA]) qualitative or semiquantitative; hepatitis B surface antigen (HBsAg) neutralization
87380	Infectious agent antigen detection by immunoassay technique, (eg, enzyme immunoassay [EIA], enzyme-linked immunosorbent assay [ELISA], fluorescence immunoassay [FIA], immunochemiluminometric assay [IMCA]) qualitative or semiquantitative; hepatitis, delta agent
87516	Infectious agent detection by nucleic acid (DNA or RNA); hepatitis B virus, amplified probe technique
87517	Infectious agent detection by nucleic acid (DNA or RNA); hepatitis B virus, quantification
87520	Infectious agent detection by nucleic acid (DNA or RNA); hepatitis C, direct probe technique
87521	Infectious agent detection by nucleic acid (DNA or RNA); hepatitis C, amplified probe technique, includes reverse transcription when performed
87522	Infectious agent detection by nucleic acid (DNA or RNA); hepatitis C, quantification, includes reverse transcription when performed
87523	Infectious agent detection by nucleic acid (DNA or RNA); hepatitis D (delta), quantification, including reverse transcription, when performed
87799	Infectious agent detection by nucleic acid (DNA or RNA), not otherwise specified; quantification, each organism
87902	Infectious agent genotype analysis by nucleic acid (DNA or RNA); Hepatitis C virus
G0472	Hepatitis C antibody screening, for individual at high risk and other covered indication(s)

CPT	Code Description
G0499	Hepatitis b screening in non-pregnant, high risk individual includes hepatitis b surface antigen (hbsag), antibodies to hbsag (anti-hbs) and antibodies to hepatitis b core antigen (anti-hbc), and is followed by a neutralizing confirmatory test, when performed, only for an initially reactive hbsag result

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

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Revision History

Review Date	Summary of Changes
09/04/2024	Reviewed and Updated: Updated background, guidelines, and evidence-based scientific references. Literature review necessitated the following changes in coverage criteria: For clarity and consistency, CC9 was edited to include "qualitative". CC now reads: "9) Qualitative nucleic acid testing for HCV MEETS COVERAGE CRITERIA in any of the following situations:" Based on CDC updates, CC9b was changed from "2-17" to "2-6", now reads: "b) One time screening for perinatally exposed infants who are 2-6 months of age." New CC for hepatitis A and hepatitis D testing: "Hepatitis A 12) For individuals with signs and symptoms of acute viral hepatitis and who have tested negative for HBV and HCV, testing for IgM anti-hepatitis A (HAV) or qualitative testing for HAV RNA MEETS COVERAGE CRITERIA. 13) Quantitative nucleic acid testing for HAV viral load DOES NOT MEET COVERAGE CRITERIA. Hepatitis D 14) For individuals who have tested positive for HBV, testing for hepatitis D virus (HDV) antibody (anti-HDV) or qualitative testing for HDV RNA MEETS COVERAGE CRITERIA. 15) Quantitative nucleic acid testing for HDV viral load DOES NOT MEET COVERAGE CRITERIA." Added CPT code 86692, 86708, 86709, 87380, 87516, 87523, 87799

Human Immunodeficiency Virus (HIV)

Policy Number: AHS – M2116 – Human Immunodeficiency Virus (HIV)	Prior Policy Name and Number, as applicable: • AHS – M2116 – Plasma HIV-1 and HIV-2 RNA Quantification for HIV Infection • AHS – M2116 – Plasma HIV-1 RNA Quantification for HIV-1 Infection
Initial Presentation Date: 11/16/2015 Revision Date: 03/06/2024	

Policy Description

Human immunodeficiency virus (HIV) is an RNA retrovirus that infects human immune cells, specifically CD4 cells, causing progressive deterioration of the immune system ultimately leading to acquired immune deficiency syndrome (AIDS) characterized by susceptibility to opportunistic infections and HIV-related cancers (CDC, 2014). HIV-1 is the dominant subtype of HIV infection, but another subtype, HIV-2, is a crucial subtype in certain areas of the world, such as Western Africa (Wood, 2023).

Related Policies

Policy Number	Policy Title
AHS-G2035	Prenatal Screening (Nongenetic)
AHS-G2157	Diagnostic Testing of Common Sexually Transmitted Infections

Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) For individuals 11 to 65 years of age, initial screening for HIV infection **MEETS COVERAGE CRITERIA**.

2) For individuals 11 to 65 years of age, repeat screening for HIV infection (no less than 90 days after initial screening) **MEETS COVERAGE CRITERIA**.

3) HIV genotyping or phenotyping **MEETS COVERAGE CRITERIA** for **any** of the following situations:
   a) Prior to initiating doravirine therapy (genotyping and phenotyping is required).
   b) For individuals who have failed a course of antiviral therapy.
   c) For individuals who have suboptimal viral load reduction.
   d) For individuals who have been noncompliant with therapy.
   e) To guide treatment decisions in individuals with acute or recent infection (within the last 6 months).
   f) For antiretroviral naïve individuals entering treatment.
   g) For all HIV-infected pregnant individuals in the following situations:
      i) Before initiation of antiretroviral therapy.
      ii) For those with detectable HIV RNA levels.

4) For treatment-experienced individuals on failing regimens who are thought to have multidrug resistance, HIV phenotyping **MEETS COVERAGE CRITERIA**.

5) When the risk of HIV infection is significant and the initiation of therapy is anticipated, a baseline HIV quantification **MEETS COVERAGE CRITERIA** in any of the following situations:
   a) In an at-risk individual with persistence of borderline or equivocal serologic reactivity.
   b) In an at-risk individual with signs and symptoms of acute retroviral syndrome (characterized by fever, malaise, lymphadenopathy, and rash).

6) Plasma quantification of HIV-1 RNA or HIV-2 RNA (see Note 1) **MEETS COVERAGE CRITERIA** for **any** of the following situations:
   a) For monitoring disease progression in HIV-infected individuals.
   b) For monitoring response to antiretroviral therapy.
   c) For infants younger than 18 months born to HIV-positive mothers (antibody tests may be confounded by maternal antibodies in this time frame).
   d) For predicting maternal-fetal transmission of HIV-1 or HIV-2.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

7) Routine use of combined genotyping and phenotyping **DOES NOT MEET COVERAGE CRITERIA**.

8) Drug susceptibility phenotype prediction using genotypic comparison to known genotypic/phenotypic database **DOES NOT MEET COVERAGE CRITERIA**.

NOTES:

Note 1: Because differences in absolute HIV copy number are known to occur using different assays, plasma HIV RNA levels should be measured by the same analytical method. A change in assay method may necessitate re-establishment of a baseline.

Table of Terminology

Term	Definition
AAP	American Academy of Pediatrics
AI/A1	Strong panel support – Evidence from ≥1 RCTs published in the peer-reviewed literature or presented in abstract form at peer-reviewed scientific meetings
ACOG	American College of Obstetricians and Gynecologists
AIDS	Acquired Immune Deficiency Syndrome
AII/A2	Strong panel support - Data from well-designed nonrandomized trials or observational cohort studies with long-term clinical outcomes
AIIa	Strong panel support – Evidence from cohort or case-control studies published in the peer-reviewed literature
AIII	Strong panel support – Based on the panel's analysis of the available evidence
ART	Antiretroviral treatment (also refers in some instances to antiretroviral testing and antiretroviral therapy)
ARV	Antiretroviral
ASHM	The Australasian Society for HIV, Viral Hepatitis and Sexual Health Medicine
ATCC	American Type Culture Collection
BHIVA	British HIV Association
BII/B2	Moderate panel support - Data from well-designed nonrandomized trials or observational cohort studies with long-term clinical outcomes
BIIa	Moderate panel support – Evidence from cohort or case-control studies published in the peer-reviewed literature
BIII	Moderate panel support – Based on the panel's analysis of the available evidence
CCR5	C-C chemokine receptor type 5
CD4	Cluster of differentiation 4
CDC	Centers for Disease Control and Prevention
CIII	Limited or weak panel support – Based on the panel's analysis of the available evidence
CMS	Centers for Medicare and Medicaid
CPD	Citrate-phosphate-dextrose
CSF	Cerebrospinal fluid
CTM	COBAS TaqMan
DHHS	Department Of Health and Human Services
DNA	Deoxyribose nucleic acid
EACS	European Acquired Immune Deficiency Syndrome Clinical Society
EDTA	Ethylenediaminetetraacetic acid
FDA	Food and Drug Administration
GIS	Genotypic interpretation systems
GPP	General practice point
GT	Genotype
HIV	Human Immunodeficiency Virus
HIV-1	Human Immunodeficiency Virus, Type 1
HIV-2	Human Immunodeficiency Virus, Type 2
HIVDR	HIV drug resistance
HIVMA	HIV Medicine Association

HIV-VL	HIV viral load
IDSA	Infection Diseases Society of America
INSTI	Integrase strand transfer inhibitor
K103N	Lysine to aspartate polymorphism
LADRV	Low abundant drug resistant variant
LDT	Laboratory developed test
NAAT	Nucleic acid amplification test
NGS	Next generation sequencing
NNRTIs	Non-nucleoside reverse transcriptase inhibitors
NRTIs	Nucleoside reverse transcriptase inhibitors
NYSDOH	New York State Department of Health
PCR	Polymerase chain reaction
PEP	Postexposure prophylaxis
PIs	Protease inhibitors
PR	Protease
RAL	Raltegravir
RCT	Randomized controlled trial
RNA	Ribonucleic acid
RT	Reverse transcriptase
RT-PCR	Reverse transcriptase polymerase chain reaction
RVA	Recombinant virus assay
SMFM	Society for Maternal-Fetal Medicine
SS	Sanger sequencing
TDR	Total drug resistance
USPSTF	United States Preventive Services Task Force

Scientific Background

Human immunodeficiency virus (HIV) targets the immune system, eventually hindering the body's ability to fight infections and diseases. If not treated, an HIV infection may lead to acquired immunodeficiency syndrome (AIDS) which is a condition caused by the virus. There are two main types of HIV: HIV-1 and HIV-2; both are genetically different. HIV-1 is more common and widespread than HIV-2.

HIV-1

Human immunodeficiency virus type 1 (HIV-1) RNA in blood can be measured using qualitative or quantitative techniques. Qualitative testing is used as a screening test to identify HIV-infected individuals whereas quantitative measurement of HIV-1 viral loads in the blood is used in management and monitoring of HIV-1 infected individuals. HIV-1 RNA levels may also be used to establish the diagnosis of HIV infection in specific situations where combination tests that detect HIV p24 antigen and HIV antibodies are not appropriate (neonatal or acute infection) (Caliendo, 2022).

Three primary real-time reverse transcriptase polymerase chain reaction (RT-PCR) commercial tests are commonly used to quantify HIV-1 RNA from plasma. These tests are more sensitive (detecting 20 to 40 copies/mL of HIV RNA), have a broader linear range (detecting virus to at least 10 million copies/mL), and pose a lower risk of carry over contamination than prior PCR assays. The tests are "COBAS AmpliPrep/ TaqMan HIV-1 Test version 2" by Roche Diagnostics, "RealTime HIV-1" and the Alinity m HIV-1 test (both by Abbott Molecular), and "Aptima HIV-1 Quant Dx Assay" by Hologic (Caliendo, 2022). In 2020, the Aptima assay received FDA approval to aid in diagnosis, in addition to its original use of quantitation (BusinessWire, 2020; FDA, 2020).

Sources of variability between HIV-1 assays include differences in technology platform, plasma input volume, and ability to detect HIV-1 subtypes. Monitoring of individual patients should be performed on the same technology platform to ensure appropriate interpretation of changes in viral load (Sollis et al., 2014). An important difference between assays is the gene target; with the increasing use of integrase inhibitors, monitoring for resistance mutations in the integrase gene is essential to ensure that the primer and probe binding sites are not impacted (Caliendo, 2022).

Overall, studies of real-time RT-PCR tests have shown high concordance, high correlation values, and good agreement among all assays (Mor et al., 2015). However, their manufacturers have reported that variation and error tend to increase at the lower limits of quantitation of the assays (Swenson et al., 2014). The high variability around the threshold of detectability of the viral load assays should be noted since many patients have viral loads in this range. Agreement between these assays was improved using a 200-copies/ml threshold (Swenson et al., 2014) consistent with the current HIV treatment guidelines' definition of virological failure (Saag et al., 2020).

Furthermore, changes in HIV-1 RNA levels must exceed at least 0.5 $\log_{10}$ or threefold in magnitude to represent biologically relevant changes in viral replication (Hughes et al., 1997; Saag et al., 1996). Viral RNA levels can also transiently rise due to acute illness, herpes outbreak, or vaccination; however, values usually return to baseline within one month (Caliendo, 2022). CD4 cell counts are weakly correlated with viral RNA measurements. Viral RNA measurements, although, do not replace CD4 cell counts in the management of HIV-1-infected patients and should be used in parallel (Caliendo, 2022).

HIV-2

Human immunodeficiency virus type 1 (HIV-2) is another subtype of HIV. Compared to HIV-1, HIV-2 appears milder clinically; it is characterized by a longer asymptomatic stage, slower declines of CD4 cell counts, and lower levels of plasma viremia in chronically ill patients (Gottlieb, 2023b). However, these numerical thresholds are not as well-defined as those of HIV-1 as there is currently not as much data available for HIV-2. Further, although quantification of HIV-2 RNA viral load may be useful, it is not widely commercially available, as the few labs that offer HIV-2 testing only offer qualitative testing and not quantitative (Gottlieb, 2023a). This is particularly crucial as HIV-1 assays typically do not properly detect HIV-2 viral load (DHHS, 2023a). It is possible for commercially available HIV-1 diagnostic assays to cross-react with HIV-2, disrupting the results. A reactive HIV-1 Western Blot may not be indicative of a true HIV-1 infection. For example, a patient may have reactive HIV serology, but test negative on a confirmatory HIV-1 Western blot. This scenario may indicate an HIV-2 infection. Clinical manifestations of HIV-2 infection are generally similar to HIV-1 infection, but much remains to be discovered about the general course of HIV-2 infection (Gottlieb, 2023a).

Despite HIV-2's milder symptoms, certain clinical features may make an infection more difficult to manage; for example, HIV-2 is intrinsically resistant to non-nucleoside reverse transcriptase inhibitors, as well as enfuvirtide. Assessment of genotypic or phenotypic resistance is also unexplored, with no currently FDA-approved genotypic or phenotypic resistance assays available (DHHS, 2023a).

Although HIV-2 is endemic to West Africa (Senegal, Gambia, Guinea-Bissau, et al.) the epidemiological trends may be shifting; the CDC only reported 166 cases of HIV-2 from 1987 to 2009 but this may be underestimated as HIV-2 is often asymptomatic. There were 24 cases of HIV-2 identified in New York City between 2010 and 2020, with 25 additional probable cases. Additionally, as much as 5% of HIV cases are thought to be HIV-2 (Gottlieb, 2023b; Quinn, 2022).

Drug Resistance

HIV replicates rapidly; a replication cycle rate of approximately one to two days ensures that after a single year, the virus in an infected individual may be 200 to 300 generations removed from the initial infection-causing virus (Coffin & Swanstrom, 2013). This leads to great genetic diversity of each HIV infection in an individual. As an RNA retrovirus, HIV requires the use of a reverse transcriptase for replication purposes. A reverse transcriptase is an enzyme which generates complimentary DNA from an RNA template. This enzyme is error-prone with the overall single-step point mutation rate reaching about 3.4×10^{-5} mutations per base per replication cycle (Mansky & Temin, 1995), leading to approximately one genome in three containing a mutation after each round of replication (some of which confer drug resistance). This rate is comparable to other RNA viruses. This pace of replication, duration of infection, and size of the replicating population allows the retrovirus to evolve rapidly in response to selective influences (Coffin & Swanstrom, 2013).

Due to the high rate of mutation in HIV viruses, drug resistance mutations are common. Some drugs may be resisted by a single mutation—these drugs have a "low genetic barrier" to resistance. Such mutations are common enough to be termed "signature mutations," which are frequently associated with a specific drug resistance. For example, the K103N mutation commonly leads to resistance for efavirenz. Efavirenz is a standard retroviral medication used to treat and prevent HIV and AIDs. To combat this, medical professionals can now assess drug-resistant HIV variants using phenotypic testing and genotypic testing (Kozal, 2019a).

Genotypic assays detect the presence of specific drug-resistance mutations in several different genes (protease, reverse transcriptase, and integrase genes). For example, assays may test for resistance in nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), or protease inhibitors (PIs). The definition of a resistance conferring mutation is blurred, but generally includes one or more of the following conditions:

- The mutation confers phenotypic resistance when introduced into a drug-sensitive laboratory strain of HIV.
- The mutation is selected for during serial in vitro passage of the virus in the presence of a drug.
- The mutation is selected for during clinical therapy with that drug.
- The presence of the mutation in clinical isolates is associated with phenotypic resistance and virologic failure (Kozal, 2019b).

Interpretation of genotypic data may be done either by clinical expertise or through a database (in which the genotype is correlated with the phenotype). Phenotypic resistance assays measure the extent to which an antiretroviral drug inhibits viral replication. Phenotypic testing typically assesses the fold-change in susceptibility of a patient's virus and the treatment response, while also correlating the mutations present with the fold-change in susceptibility. Recombinant virus assays (RVAs) are used; protease, reverse transcriptase, or integrase gene sequences from circulating viruses are inserted into a reference strain of HIV, and this new HIV strain is measured by the phenotypic assay (Kozal, 2019b).

Several HIV genotypic assays are available. The ViroSeq HIV-1 Genotyping system by Abbott helps to detect HIV-1 genomic mutations that may lead to resistance to certain types of antiretroviral drugs (ThermoFisher, 2011). The ATCC® HIV-1 Drug Resistance Genotyping Kit has been developed by the American Type Culture Collection (ATCC), the Centers for Disease Control and Prevention (CDC) and Thermo Fischer Scientific; this is a real time- polymerase chain reaction (rt-PCR) assay which may help to identify and monitor HIV-1 drug resistance (ATCC, 2014).

The primary phenotypic assay is "PhenoSense" from LabCorp. The Human Immunodeficiency Virus 1 (HIV-1) PhenoSense GT® Plus Integrase (Monogram® Phenotype + Genotype) test by LabCorp measures HIV genotypic and phenotypic resistance from plasma samples (LabCorp, 2021).

Advantages of the genotype assays include lower cost and shorter turnaround time. However, interpretation of these assays is complicated by combinations of individual mutations that may have a differential effect on resistance that differs from the individual mutation alone (Kozal, 2019b). Mutation combinations are known to cause resistance to certain drugs, but increase susceptibility to others, impact viral fitness, and contribute to major pathways of resistance; additionally, the interactions of mutations affecting various mechanisms can be difficult to predict. Over 20 rules-based genotypic interpretation systems (GIS) have been proposed (Fox et al., 2007; Kozal, 2019b).

Advantages of phenotypic assays include an ability to measure resistance more directly and examine the relative effect of multiple mutations on drug resistance. Limitations of the phenotypic assays include a longer turnaround time, greater expense, and biologic cut-offs above achievable drug levels. Phenotypic resistance assays may be helpful when evaluating HIV strains with known or suspected complex drug resistance mutation patterns as their actual resistance may not be accurately predicted by simply detecting the presence of multiple mutations (Kozal, 2019b). Both assays are limited by decreased sensitivity for low-level minority variants that comprise less than five to 20 percent of the virus population (Kozal, 2019b).

Analytical Validity

Rosemary et al. (2018) performed a comparison of two genotyping assays, ViroSeq and ATCC (manufactured by Thermo-Fisher Scientific) kit. A total of 183 samples with a viral load ≥1000 copies/mL were sequenced by ViroSeq and randomly selected (85 successfully genotyped, 98 unsuccessfully genotyped). The ATCC kit also genotyped 115 of the 183 samples, and out of the 98 unsuccessfully genotyped samples, the ATCC kit was able to genotype 42. Overall, 127 of the 183 samples were genotyped. The authors noted that the sequences of the genotyped samples were 98% identical and had "similar HIVDR profiles at individual patient level" (Rosemary et al., 2018).

Braun et al. (2020) evaluated the diagnostic performance and analytical validity of the Alinity m HIV-1 assay, a test which uses a dual target and dual probe "against the highly conserved target regions of the HIV-1 genome." As part of the international and multisite study, Alinity m was compared with four other commercially available tests. The Alinity assay performed comparatively to currently available HIV-1 tests with "comparable detection of 16 different HIV-1 subtypes (R2 = 0.956). A high level of agreement (>88 %) between all HIV-1 assays was seen near clinical decision points of 1.7 Log10 copies/mL (50 copies/mL) and 2.0 Log10 copies/mL (200 copies/mL)." Additionally, a high level of detectability (≥97 % hit rate) was shown with reproducibility across sites (Braun et al., 2020).

Clinical Utility and Validity

Zhang et al. (2005) compared two phenotyping assays, Antivirogram and PhenoSense. Reverse transcriptase inhibitor susceptibility results were evaluated for 202 isolates from Antivirogram and 126 from PhenoSense. The authors found the median deviance for wild-type and mutant isolates to be lower for PhenoSense compared to Antivirogram, and PhenoSense was more likely to detect resistance to abacavir, didanosine, and stavudine when common drug resistance mutations were present (Zhang et al., 2005).

Hopkins et al. (2015) performed a study comparing the three main RT-PCR tests available, Aptima,

COBAS TaqMan (CTM), and Abbott RealTime. The assays were evaluated based on plasma samples from 191 HIV positive patients as well as WHO International Standards (12-500 copies/mL). Aptima detected 141/191 (74%) of the HIV samples, CTM detected 145/191 (76%), and Abbott RealTime detected 119/191 (62%). The authors noted that precision decreased as the viral load got closer to the lower limit of quantification of 50 copies/mL (Hopkins et al., 2015).

Sempa et al. (2016) evaluated the utility of HIV-1 viral load as a prognostic indicator. A total of 489 patients were evaluated, and the viral load curves were evaluated on a linear scale and a logarithmic scale. The authors found that the viral load curve on the logarithmic scale was a statistically significant predictor of mortality, noting that each log10 increase in viral load corresponded to a 1.63 times higher risk of mortality. However, the authors stress that the choice of variables and statistical model influences the predictive power of this metric (Sempa et al., 2016).

Shen et al. (2016) assessed the ability to predict phenotypic drug resistance from genotypic data. The authors used two machine learning algorithms to predict drug resistance to HIV protease inhibitors and reverse transcriptase inhibitors as well as the severity of that resistance from a query sequence. The accuracy of these classifications was found to be >0.973 for eight PR inhibitors and 0.986 for ten RT inhibitors and the r^2 was 0.772–0.953 for the PR cohort and 0.773–0.995 for the RT cohort. The algorithms' results were verified by "five-fold cross validation" on the genotype-phenotype datasets (Shen et al., 2016).

Lindman et al. (2019) investigated the test performance of the Bio-Rad Geenius HIV-1/2 confirmatory assay against INNO-LIA HIV 1/2 Score and ImmunoComb HIV 1/2 BiSpot. The Geenius test is purported to differentiate between HIV-1 and HIV-2 infections. 131 samples from ART naïve HIV infected patients in Guinea-Bissau were evaluated. The Geenius test identified 62 samples as "HIV-1 reactive", 37 as "HIV-2 reactive" and 32 as "HIV-1/2 dually reactive." INNO-LIA identified 63 as HIV-1 reactive, 36 as HIV-2 reactive, and 32 as HIV-1/2 dually reactive. The agreement between Geenius compared to INNO-LIA and Immunocomb was 92.4% and 84% respectively (Lindman et al., 2019).

Avram et al. (2019) compared the cost-effectiveness of measuring viral load to guide delivery in HIV-positive women and compared it to routine cesarian delivery. A theoretical cohort of 1275 women was used, and the authors produced a decision-analytic model to compare the two techniques. The average cost of a point-of-care HIV RNA viral load test was placed at $15.22. The authors also assumed that each woman in the cohort would deliver two children. The authors defined the primary outcomes as "mother-to-child transmission, delivery mode, cesarean delivery-related complications, cost, and quality-adjusted life years", and the cost-effectiveness threshold was $100,000/quality-adjusted life year. The authors found that measuring viral load resulted in more HIV-infected neonates than routine cesarian delivery for all due to "viral exposure during more frequent vaginal births in this strategy." The authors found an increased cost of $3,883,371 and decreased quality-adjusted life years of 63 in the measurement strategy compared to the routine cesarian delivery strategy. At $100,000/quality-adjusted life year, measuring viral load was found to be cost-effective only "when the vertical transmission rate in women with high viral load below 0.68%" (compared to a baseline of 16.8%) and "when the odds ratio of vertical transmission with routine cesarean delivery for all compared with vaginal delivery was above 0.885" (compared to a baseline of 0.3). The authors concluded that "for HIV-infected pregnant women without prenatal care, quantifying viral load to guide mode of delivery using a point-of-care test resulted in increased costs and decreased effectiveness when compared with routine cesarean delivery for all, even after including downstream complications of cesarean delivery" (Avram et al., 2019).

Raymond et al. (2020) evaluated the performance of the Vela Dx Sentosa next-generation sequencing (NGS) system for HIV-1 DNA genotypic resistance. 40 DNA samples were analyzed with Vela Dx Sentosa assay and the results were compared with Sanger sequencing. The Vela Dx Sentosa assay was 100% successful in amplifying and sequencing the protease and reverse transcriptase, and 86% successful in amplifying integrase sequences when the HIV DNA load was greater than 2.5 log copies/million cells. The Sentosa and Sanger sequencing were concordant for predicting protease-reverse transcriptase resistance in 20% of the 14/18 samples which were successfully sequenced. Sentosa was able to predict a higher level of resistance in three of the samples. The Vela Dx Sentosa predicted the prevalence of drug resistance to protease inhibitors (7%), nucleoside reverse transcriptase inhibitor (59%), nonnucleoside reverse transcriptase inhibitor (31%), and integrase inhibitors (20%). Overall, the authors conclude that the Vela Dx Sentosa assay can accurately predict HIV DNA drug resistance (Raymond et al., 2020).

Fogel et al. (2020) also analyzed the ability of next-generation sequencing methods to analyze HIV drug resistance. In this case, 145 plasma samples were analyzed using the ViroSeq HIV-1 Genotyping System and the veSEQ-HIV assay. Results were compared with the Abbott RealTime Viral Load assay. 142 HIV protease and reverse transcriptase sequences and 138 integrase sequences were obtained with ViroSeq. On the other hand, veSEQ-HIV detected 70.4% of the samples with protease, reverse transcriptase, and integrase sequences. Drug resistance mutations were detected in 33 ViroSeq samples and 42 veSEQ-HIV samples. Overall, veSEQ-HIV predicted more drug resistance mutations and worked better for larger viral loads. Results from veSEQ-HIV strongly correlated with the results from Abbott RealTime Viral Load assay. The authors conclude that the veSEQ-HIV assay provided results for most samples with higher viral loads, was accurate for predicting drug resistance mutations, but detected mutations at lower levels compared with the ViroSeq assay (Fogel et al., 2020).

Pröll et al. (2022) investigated whether NGS from proviral DNA and RNA could be an alternative to using plasma viral RNA as the material of choice for genotypic resistance testing at the start of ART and virologic failure for patients with low viremia. When taking samples from 36 patients, with varying viral loads of 96 to 390,000 copies/mL, the researchers found 2476 variants/drug resistance mutations by SS, while 2892 variants were found by NGS. Researchers stated, "An average of 822/1008 variants were identified in plasma viral RNA by Sanger or NGS sequencing, 834/956 in cellular viral RNA, and 820/928 in cellular viral DNA." This demonstrates that cellular RNA and cellular viral DNA could serve as viable substitutes when testing for variant detection and genotypic resistance among patients with HIV and low viremia (Pröll et al., 2022).

Ehret et al. (2022) tested the performance of the "Xpert® HIV-1 Viral Load (VL) XC" HIV-RNA quantitative assay made by Cepheid. This assay has been redesigned to use a dual-target approach. The authors tested 533 fresh and frozen samples from HIV-1 positive patients on the Abbott HIV-assay and the Xpert XC assay. "The Xpert XC assay yielded valid results in 98.5% (N = 528/536) of cases." The authors conclude that "The Xpert XC assay showed excellent correlation with the Abbott assays for all tested HIV-1 subtypes" (Ehret et al., 2022).

Guidelines and Recommendations

Department of Health and Human Services (DHHS)

The Department of Health and Human Services (DHHS) Panel on Antiretroviral Guidelines for Adults and Adolescents updated the guidelines on use of antiretroviral drugs in 2022. The panel states "viral load is the most important indicator of initial and sustained response to ART and should be measured in all

patients with HIV at entry into care (AI), at initiation of therapy (AI), and on a regular basis thereafter. For those patients who choose to delay therapy or remain untreated for whatever reason, repeat viral load testing while not on ART is optional (CIII). Pre-treatment viral load level is also an important factor in the selection of an initial ARV regimen, because several currently approved ARV drugs or regimens have been associated with poorer responses in patients with high baseline viral load."

The panel's recommendations on the frequency of viral load monitoring are summarized below (DHHS, 2023a):

- "After initiation of ART: Plasma viral load should be measured before initiation of ART and within 4 to 8 weeks after treatment initiation (AIII). The purpose of the measurements is to confirm an adequate virologic response to ART, indicating appropriate regimen selection and patient adherence to therapy. Repeat viral load measurement should be performed at 4- to 8-week intervals until the level falls below the assay's limit of detection (BIII)."
- "In patients with viral suppression, with ART modification because of drug toxicity or for regimen simplification: Viral load measurement should be performed within 4 to 8 weeks after changing therapy (AIII). The purpose of viral load monitoring at this point is to confirm the effectiveness of the new regimen."
- "In patients on a stable, suppressive ARV regimen: Viral load measurement should be repeated every 3 to 4 months (AIII) or as clinically indicated to confirm continuous viral suppression. Clinicians may extend the interval to 6 months for adherent patients whose viral load has been suppressed for more than a year, whose clinical and immunologic status is stable, and who are not at risk for inadequate adherence (AIII)."
- "In patients with virologic failure who require a change in ARV regimen: Plasma viral load should be measured before ART change and within 4 to 8 weeks after treatment modification (AIII). The purpose of the measurements is to confirm an adequate virologic response to the new regimen. Repeat viral load measurement should be performed at 4- to 8-week intervals until the level falls below the assay's limit of detection (BIII). If viral suppression is not possible, repeat viral load measurement every 3 months or more frequently if indicated (AIII)."
- "In patients with suboptimal response: The frequency of viral load monitoring will depend on clinical circumstances, such as adherence and availability of further treatment options. In addition to viral load monitoring, several other factors—such as patient adherence to prescribed medications, suboptimal drug exposure, or drug interactions—should be assessed. Patients who fail to achieve viral suppression should undergo drug-resistance testing to aid in the selection of an alternative ARV regimen."

The guideline also comments on HIV-2. Although the optimal treatment strategy has not been defined, the guideline does recommend that quantitative plasma HIV-2 RNA viral load testing should be performed before initiating ART (AIII). HIV-2 RNA should also be used to assess treatment response. The guideline also notes that the "Geenius HIV 1/2 Supplemental Assay (Bio-Rad Laboratories)" is FDA-approved to differentiate HIV-1 infection from HIV-2 infection (DHHS, 2023a).

In an updated review in 2022, the DHHS also strongly recommended (AIII) hat "A blood sample for genotypic resistance testing should be sent to the laboratory before initiation of ART." Moreover, "Pregnancy testing should be performed in persons of childbearing potential before initiation of ART."

The DHHS propounds further, stating the following:

- "Combination immunoassays that detect HIV-1 and HIV-2 antibodies and HIV p24 antigen (Ag/Ab assays) are part of the recommended initial laboratory HIV testing algorithm, primarily due to

their enhanced ability to detect acute HIV infection. Specimens that are reactive on an initial Ag/Ab assay should be tested with an immunoassay that differentiates HIV-1 from HIV-2 antibodies. Specimens that are reactive on the initial assay and have either negative or indeterminate antibody differentiation test result should be tested for quantitative or qualitative HIV RNA; an undetectable HIV RNA test result indicates that the original Ag/Ab test result was a false positive. Detection of HIV RNA in this setting indicates that acute HIV infection is highly likely."

- "HIV infection should be confirmed by repeat quantitative HIV RNA testing or subsequent testing to document HIV antibody seroconversion."
- "The proposed threshold of <3,000 copies/mL is based on historical data that used laboratory methods that are now considered obsolete. These older viral load assays demonstrated false-positive cases of acute HIV infection at HIV RNA levels of <3,000 copies/mL. However, improvements in plasma viral load methodology suggest that any positive result on a quantitative plasma HIV RNA test in the setting of a negative or indeterminate antibody test result is highly consistent with acute HIV infection, including at HIV RNA levels of <3,000 copies/mL. HIV RNA levels in acute infection are generally very high (e.g., >100,000 copies/mL); however, levels may be <3,000 copies/mL in the earliest weeks following infection as viral load continues to rise. Therefore, when a low-positive quantitative HIV RNA test result is present at this level, the HIV RNA test should be repeated on a new blood specimen to confirm the diagnosis. Repeated false-positive HIV RNA test results are unlikely" (DHHS, 2023a).

As persons who acquire HIV while taking pre-exposure prophylaxis (PrEP) may present ambiguous HIV test results, the DHHS proposes that:

- "A positive HIV Ag/Ab test result or a positive HIV RNA test result in the setting of a negative HIV antibody test result should prompt immediate confirmation of HIV diagnosis. It is important to collect a new blood specimen to verify the HIV diagnosis before initiating HIV treatment."
- "In people with HIV RNA level ≥200 copies/mL who are taking PrEP, immediate initiation of an effective HIV treatment regimen is recommended while awaiting confirmation of HIV diagnosis (AIII)."
- "In people taking PrEP who have a negative HIV antibody test result and a very low-positive quantitative HIV RNA test result (<200 copies/mL) a confirmatory HIV antibody test and repeat quantitative plasma HIV RNA test should be performed, and results should be available before initiating ART."
- "In rare cases, particularly when PrEP is transitioned to an ARV regimen and HIV RNA and antibody diagnostic testing are inconclusive, HIV DNA testing may be of value" (DHHS, 2023a).

The Department of Health and Human Services (DHHS, 2023a, 2023b, 2023c) updated their guidelines for using drug resistance assays in HIV infections. The guidelines recommend HIV genotyping or phenotyping in the following situations among pregnant individuals and reducing perinatal HIV transmission in the US:

- "General Principles Regarding Use of Antiretroviral Drugs During Pregnancy:
    - Antiretroviral (ARV) drug-resistance genotype evaluations or assays should be performed before starting ARV drug regimens in people who are ARV-naive (AII) or ARV-experienced (AIII) and before modifying ARV drug regimens (AII) in people whose HIV RNA levels are above the threshold for resistance testing (i.e., >500 copies/mL to 1,000 copies/mL).
    - In pregnant people who are not already receiving ART, ART should be initiated before results of drug resistance testing are available because earlier viral suppression has been associated

with lower risk of transmission. When ART is initiated before results are available, the regimen should be modified, if necessary, based on resistance assay results (AII)."

- "Pregnant People with HIV Who Have Never Received Antiretroviral Drugs (Antiretroviral Naive)
  - For pregnant people who have never received antiretroviral therapy (ART), ART should be initiated as soon as possible, even before results of drug-resistance testing are available, as viral suppression earlier in pregnancy has been associated with lower risk of transmission (AI). When ART is initiated before the results of the drug resistance assays are available, the ARV regimen should be modified, if necessary, based on the resistance assay results (AII)."
- "People with HIV Who Are Taking Antiretroviral Therapy When They Became Pregnant
  - For pregnant people on ART, ARV drug-resistance testing should be performed to assist the selection of active drugs when changing ARV regimens in pregnant people who are experiencing virologic failure on ART and who have HIV RNA levels >500 copies/mL to 1,000 copies/mL (AII). In individuals who have HIV RNA levels >500 copies/mL but <1,000 copies/mL, testing may be unsuccessful but still should be considered (BII)."
- "Pregnant People with HIV Who Have Previously Received Antiretroviral Medications but Are Not Currently Receiving Any Antiretroviral Medications
  - If HIV RNA is above the threshold for standard genotypic drug resistance testing (i.e., >500 to 1,000 copies/mL), ARV drug-resistance testing should be performed prior to starting an ARV drug regiment (AIII)
  - ART should be initiated prior to receiving results of current ARV resistance assays. ART should be modified based on the results of the resistance assay, if necessary (AII)."
- "Initial Evaluation and Continued Monitoring of HIV-Related Assessments During Pregnancy
  - HIV drug-resistance testing (genotypic testing and, if indicated, phenotypic testing) should be performed during pregnancy in those whose HIV RNA levels are above the threshold for resistance testing (i.e., >500 copies/mL to 1,000 copies/mL) before –
    - Initiating ART in antiretroviral (ARV)-naive pregnant people who have not been previously tested for ARV drug resistance (AII);
    - Initiating ART in ARV-experienced pregnant people (including those who have received preexposure prophylaxis) (AIII); or
    - Modifying ARV regimens for people with HIV who become pregnant while receiving ARV drugs or people who have suboptimal virologic response to ARV drugs that were started during pregnancy (AII).
  - ART should be initiated in pregnant patients prior to receiving the results of ARV-resistance tests. ART should be modified, if necessary, based on the results of resistance testing (AII)."
- "Antiretroviral Drug Resistance and Resistance Testing in Pregnancy
  - HIV drug-resistance testing (genotypic and, if indicated, phenotypic) should be performed in persons living with HIV whose HIV RNA levels are above the threshold for resistance testing (i.e., >200 to 1,000 copies/mL). For people with confirmed HIV RNA levels >200 copies/mL but <1,000 copies/mL, drug-resistance testing may be unsuccessful but should still be considered. Perform resistance testing before:
    - Initiating ART in ARV-naive pregnant women who have not been previously tested for ARV resistance (AII),
    - initiating ART in ARV-experienced pregnant women (including those who have received pre-exposure prophylaxis) (AIII), *or*
    - modifying ART regimens for those who are newly pregnant and receiving ARV drugs or who have suboptimal virologic response to the ARV drugs during pregnancy (AII).
  - Phenotypic resistance testing is indicated for treatment-experienced persons on failing regimens who are thought to have multidrug resistance (BIII).

- ART should be initiated in pregnant persons before receiving results of ARV-resistance testing; ART should be modified, if necessary, based on the results of resistance assays (AII).
- If the use of an integrase strand transfer inhibitor (INSTI) is being considered and INSTI resistance is a concern, providers should supplement standard resistance testing with a specific INSTI genotypic resistance assay (AIII). INSTI resistance may be a concern if-
  - a patient received prior treatment or pre-exposure prophylaxis (PrEP) that included an INSTI, *or*
  - a patient has a history with a sexual partner on INSTI therapy who was not virologically suppressed or with unknown viral load" (DHHS, 2023c).

Among adults and adolescents living with HIV, the DHHS recommends the following for drug resistance testing:

- "For initial treatment:
  - HIV drug-resistance testing is recommended at entry into care for persons with HIV to guide selection of the initial antiretroviral therapy (ART) regimen (AII). If therapy is deferred, repeat testing may be considered at the time of ART initiation (CIII)
  - Genotypic, rather than phenotypic, testing is the preferred resistance testing to guide therapy in antiretroviral (ARV)-naive patients (AIII)
  - In persons with acute or recent (early) HIV infection, in pregnant people with HIV, or in people who will initiate ART on the day of or soon after HIV diagnosis, ART initiation should not be delayed while awaiting resistance testing results; the regimen can be modified once results are reported (AIII)
  - Standard genotypic drug-resistance testing in ARV-naive persons involves testing for mutations in the reverse transcriptase and protease genes. If transmitted integrase strand transfer inhibitor (INSTI) resistance is suspected or if the person has used long-acting cabotegravir (CAB-LA) as pre-exposure prophylaxis (PrEP) in the past, providers should ensure that genotypic resistance testing also includes the integrase gene (AIII).
- For Antiretroviral Therapy-Experienced Persons:
  - HIV drug-resistance testing should be performed to assist the selection of active drugs when changing ART regimens in the following patients:
    - People with virologic failure and HIV-RNA levels >200 copies/mL (AI for >1,000 copies/mL, AIII for 501–1,000 copies/mL, CIII for confirmed HIV RNA 201–500 copies/mL). For people with confirmed HIV-RNA levels >200 copies/mL but >500 copies/mL, drug-resistance testing may be unsuccessful but should still be considered.
    - Persons with suboptimal viral load reduction (AII)
  - Reverse transcriptase and protease genotypic resistance testing should be performed on everyone with virologic failure; integrase resistance testing (which may need to be ordered separately) should be performed on individuals experiencing virologic failure while receiving an INSTI-based regimen (AII).
  - For persons taking a non–long-acting ARV regimen, drug-resistance testing in the setting of virologic failure should be performed while the person is still taking their ARV regimen or, if that is not possible, within 4 weeks after discontinuing their ARV regimen (AII). If more than 4 weeks have elapsed since the non–long-acting agents were discontinued, resistance testing may still provide useful information to guide therapy; however, it is important to recognize that previously-selected resistance mutations can be missed due to lack of drug-selective pressure (CIII).
  - Given the long half-lives of the long-acting injectable ARV drugs, resistance testing (including testing for resistance to INSTIs) should be performed in all persons who have experienced

virologic failure on a regimen of long-acting CAB and rilpivirine or acquired HIV after receiving CAB-LA as PrEP, regardless of the amount of time since drug discontinuation (AIII).
    - Genotypic testing is preferred over phenotypic-resistance testing to guide therapy in people with suboptimal virologic response or virologic failure while on first- or second-line regimens and in people in whom resistance mutation patterns are known or not expected to be complex (AII).
    - The addition of phenotypic- to genotypic resistance testing is recommended for people with known or suspected complex drug-resistance mutation patterns (BIII).
    - All prior and current drug-resistance test results, when available, should be reviewed and considered when constructing a new regimen for a patient (AIII)" (DHHS, 2023a).

In terms of the usage of drug-resistance assays among adolescents and adults with HIV, the DHHS recommends the following:

- "In acute or recent (early) HIV infection: Drug-resistance testing is recommended (AII). A genotypic assay is generally preferred (AIII). Treatment should not be delayed while awaiting results of resistance testing (AIII).
    - If ART is deferred, repeat resistance testing may be considered when therapy is initiated (CIII). A genotypic assay is generally preferred (AIII)."
- "In ART-naive patients with chronic HIV: Drug-resistance testing is recommended at entry into HIV care to guide selection of initial ART (AII). A genotypic assay is generally preferred."
    - For pregnant persons, or if ART will be initiated on the day of or soon after HIV diagnosis, treatment can be initiated prior to receiving resistance testing results.
    - If an INSTI is considered for an ART-naïve patient and/or transmitted INSTI resistance is a concern, providers should supplement standard resistance testing with a specific INSTI genotypic resistance assay, which may need to be ordered separately (AIII).
    - If therapy is deferred, repeat resistance testing may be considered when therapy is initiated (CIII). A genotypic assay is generally preferred (AIII)."
- "In patients with virologic failure: Drug-resistance testing is recommended in patients on combination ART with HIV-RNA levels >200 copies/mL (AI for >1,000 copies/mL, AIII for 501–1,000 copies/mL) and a confirmed HIV RNA 201–500 copies/mL (CIII). In patients with confirmed HIV-RNA levels between 201–500 copies/mL, testing may not be successful but should still be considered."
    - "Resistance testing should be done while the patient is taking ART or, if that is not possible, within 4 weeks after discontinuation of non–long-acting ARV drugs (AII). If >4 weeks have elapsed, resistance testing may still be useful to guide therapy; however, previously-selected mutations can be missed due to lack of drug-selective pressure (CIII)."
    - "A standard genotypic resistance assay is generally preferred for patients experiencing virologic failure on their first or second ARV regimens and for those with expected noncomplex resistance patterns (AII)"
    - "All prior and current drug-resistance testing results should be reviewed and considered when designing a new ARV"
    - "When virologic failure occurs in a patient on an INSTI-based regimen or in a patient with a history of INSTI use, genotypic testing for INSTI resistance should be performed to determine whether to include drugs from this class in subsequent regimens (AII)."
    - "Adding phenotypic testing to genotypic testing is generally preferred in patients with known or suspected complex drug-resistance patterns (BIII)"

- "In patients with suboptimal suppression of viral load: Drug-resistance testing is recommended in patients with suboptimal viral load suppression after initiation of ART (AII)"
- "In Pregnant People with HIV: Genotypic resistance testing is recommended for all pregnant people before initiation of ART (AIII) and for those entering pregnancy with detectable HIV-RNA levels while on therapy (AI)."
- "In Patients with Undetectable Viral Load or Low-Level Viremia Who Are Planning to Change Their ARV Regimen HIV-1: proviral DNA resistance assays may be useful in patients with HIV RNA below the limit of detection or with low-level viremia, where a HIV-RNA genotypic assay is unlikely to be successful (CIII)" (DHHS, 2023a).

The DHHS also added guidelines on genotypic and phenotypic testing for pediatric HIV infection:

- "Antiretroviral (ARV) drug-resistance testing is recommended at the time of HIV diagnosis, before initiation of therapy, in all ART naive patients, and before switching regimens in patients with treatment failure (AII). Genotypic resistance testing is preferred for this purpose (AIII)."
- "Phenotypic resistance testing should be considered (usually in addition to genotypic resistance testing) for patients with known or suspected complex drug resistance mutation patterns, which generally arise after a patient has experienced virologic failure on multiple ARV regimens (CIII)" (DHHS, 2023b).

International Antiviral Society

The International Antiviral Society published a 2022 update titled "Antiretroviral Drugs for Treatment and Prevention of HIV Infection in Adults." The guideline also recommends laboratory testing to "characterize" the HIV stage prior to starting antiretroviral testing (ART); this is done by assessing HIV RNA level (Gandhi et al., 2022).

The guideline also remarks on the frequency of testing during ART. Their recommendations are as follows:

- "Within 6 weeks of starting ART, assessment of treatment adherence and tolerability is recommended, along with the measurement of HIV RNA level."
- "If the HIV RNA level has not declined by 2 log10 copies/mL within 12 weeks of therapy and adherence appears to be sufficient, then a genotype based on the patient's regimen is recommended."
- "If the patient remains virally suppressed, clinically stable, and adherent to medications, then HIV RNA levels should be monitored every 3 months until virally suppressed for at least 1 year. Afterward, the frequency of viral monitoring can be changed to every 6 months."
- "If HIV RNA level is greater than 200 copies/mL on 2 consecutive measurements, then HIV RT-pro genotype and InSTI [in integrase strand transfer inhibitor] genotype (if the patient was receiving an InSTI) testing are recommended."
- "For patients with intermittent or persistent low-level viremia between 50 and 200 copies/mL, assessments for ART adherence, tolerability, and toxic effects are recommended, but changing ART regimens is not recommended unless ART toxicity or intolerability are identified" (Gandhi et al., 2022).

On resistance test, the 2022 update notes that, "in persons diagnosed with HIV while receiving TXF-based PrEP, resistance testing should be performed but initiation of ART need not be delayed while awaiting genotype results." The panel further recommends:

- "Unless there is documented or suspected history of treatment failure, proviral resistance testing is not required prior to switching to 2-drug therapy, even if there is no available pretreatment resistance test result."
- "For patients who have maintained viral suppression, switching from long-acting injectable cabotegravir plus rilpivirine back to daily oral therapy can be done without the need for proviral DNA resistance testing."
- "If virologic failure is confirmed, genotype resistance testing should be performed, preferably while patients are taking the failing therapy. Resistance testing is still recommended even if a regimen has been discontinued or a person acknowledges poor medication adherence" (Gandhi et al., 2022).

Infectious Diseases Society of America (IDSA)

The IDSA recommends that "A quantitative HIV RNA (viral load) level should be obtained upon initiation of care (strong recommendation, high quality evidence)" (Thompson et al., 2020).

IDSA recommends rechecking HIV RNA after 2-4 weeks of initiating antiretroviral therapy (ART) (and no later than 8 weeks). From there, IDSA recommends "checking HIV RNA every 4-8 weeks until suppression is achieved." The IDSA also notes that viral load "should" be monitored every 3-4 months to "confirm maintenance of suppression below the limit of assay detection," 6 months for "adherent patients whose viral load has been suppressed for more than 2 years and whose clinical and immunologic status is stable", and more frequently after initiation or change in ART (IDSA recommends within 2-4 weeks of initiation or change but not more than 8 weeks) (Thompson et al., 2020).

Overall, IDSA lists two primary uses for viral load testing; to establish baseline and to monitor viral suppression (Thompson et al., 2020).

American College of Obstetricians and Gynecologists (ACOG)

In 2014, ACOG released "Committee on Gynecologic Practice: Routine human immunodeficiency virus screening," which they reaffirmed in 2020. Regarding routine human immunodeficiency screening, "The American College of Obstetricians and Gynecologists (the College) recommends routine HIV screening for females aged 13–64 years and older women with risk factors. Screening after age 64 years is indicated if there is ongoing risk of HIV infection, as indicated by risk assessment (e.g., new sexual partners)" (ACOG, 2014).

The College also expatiates upon repeat testing, entrusting obstetrician–gynecologists to annually review patients' risk factors for HIV and assess their needs, and recommends that "HIV testing should be offered at least annually to women who

- are injection drug users
- are sex partners of injection-drug users
- exchange sex for money or drugs
- are sex partners of HIV-infected persons
- have had sex with men who have sex with men since the most recent HIV test
- have had more than one sex partner since their most recent HIV test

The opportunity for repeat testing should be made available to all women even in the absence of identified risk factors. Repeat screening after age 64 years is indicated if there is ongoing risk of HIV infection, as indicated by an individualized risk assessment. Obstetrician–gynecologists also should

encourage women and their prospective sex partners to be tested before initiating a new sexual relationship. The benefits of periodic retesting should be discussed with patients and provided if requested, regardless of risk factors. Patients may be concerned about their status and do not know about or want to disclose risk-taking behavior to their health care providers" (ACOG, 2014).

In their 2018 committee opinion "Labor and Delivery Management of Women With Human Immunodeficiency Virus Infection," ACOG notes that current and ongoing research has shown that "treatment of HIV-infected pregnant women with combined antiretroviral therapy can achieve a 1–2% or lower risk of mother-to-child transmission if maternal viral loads of 1,000 copies/mL or less can be sustained, independent of the route of delivery or duration of ruptured membranes before delivery." ACOG further observes that "the risk of mother-to-child transmission in HIV-infected women with high viral loads can be reduced by performing cesarean deliveries before the onset of labor and before rupture of membranes [cesarean delivery in this document [the ACOG guideline]), in conjunction with the use of peripartum maternal antiretroviral therapy."

ACOG recommends offering a "scheduled prelabor cesarean delivery at 38 0/7 weeks of gestation to reduce the risk of mother-to-child transmission" if an HIV-positive pregnant woman is found to have a viral load of over 1000 copies/mL at or near delivery, independent of antepartum antiretroviral therapy. This recommendation also applies to patients whose viral load is unknown (ACOG, 2018).

Society for Maternal-Fetal Medicine (SMFM)

The SMFM published a "checklist for pregnancy management in persons with HIV." Although these checklists are not definitive, they are intended to "help ensure that all relevant elements are considered for every person with HIV during prepregnancy, antepartum, intrapartum, and postpartum periods." During the third trimester, the checklist calls for viral load to be assessed at 34-36 weeks for delivery planning (and to assess adherence and viral resistance if viral load is not suppressed). Further, if the viral load is found to be ≥1000 copies/mL at 37-38 weeks, a caesarean delivery should be scheduled for 38 weeks (Gibson & Toner, 2020).

British HIV Association

The British HIV Association (BHIVA) makes several recommendations regarding assessment of viral load during the routine investigation and/or maintenance of HIV-1 positive adults. Relevant recommendations are as follows:

- "We recommend that an HIV viral load should be performed at the first visit following serological diagnosis (1A).
- We recommend that undetectable viral load result whilst not on treatment needs repeating, review of serology to exclude HIV-2 and measurement on a different viral load assay (1D).
- We recommend a repeat HIV viral load in all new transfers prior to repeat prescriptions if it is not possible to confirm a recent viral load from the previous clinic (1A).
- We recommend that viral load measurements be taken at 1, 3 and 6 months after starting ART (1B).
- We recommend that additional viral load measurements are taken between 2 and 5 months after starting ART if viral load has not decreased at least 10-fold after 1 month of ART or there are concerns about the patient's adherence to therapy (1D).
- We recommend that viral load testing should be performed routinely every 6 months (1A) and might be at intervals of up to 12 months for patients established on ART that includes a PI (GPP)

[general practice point].

- We recommend that viral load rebound to above 50 copies/mL should be confirmed by testing a subsequent sample (2A). Repeat testing of the same sample is not recommended.
- For patients stable on ART we recommend that:
- Frequent (3–4 monthly) viral load follow-ups of individuals with stable unsuppressed (<200 copies/mL) viral loads if they are managed as low-level viraemic patients according to the BHIVA treatment guidelines (1D).
- CSF HIV viral load measurement should be considered to exclude compartmentalisation (1C)" (BHIVA, 2019).

The BHIVA released guidelines for the management of HIV-2 (BHIVA, 2021). For the diagnosis of HIV-2, the BHIV recommends:

For the diagnosis of chronic HIV-2:

- "An initial diagnosis of chronic HIV-2 infection should be made using a total of three CE-marked serology tests (i.e. tests conform to EU health and safety requirements) performed in an ISO 15189-accredited laboratory. There must be reactivity in two CE-marked fourth-generation tests for HIV-1 and HIV-2, followed by differentiation of HIV-2 by a third CE-marked antibody-only test."
- "Clinicians should consider revisiting a previous diagnosis of HIV-1 by repeating HIV-2 serology and molecular tests in individuals with an undetectable HIV-1 viral load in the absence of ART, but a falling CD4 count. This is in order to detect the possibility of missed HIV-1 and HIV-2 dual infection."
- "In those with diagnosed HIV-2 with an undetectable viral load in the absence of ART, clinicians should consider repeating HIV-1 diagnostic tests, if their CD4 count falls. This is to investigate the possibility of HIV-1 superinfection."

For the diagnosis of acute primary HIV-2

- "Investigation for acute or very recent HIV-2 infection should start as for diagnosis of chronic HIV-2 infection. A negative HIV-2 screening result on a blood sample taken within 3 months of the likely exposure should be further investigated at 6 weeks and 3 months, with parallel testing for HIV-2 viral RNA and, if necessary, HIV-2 proviral DNA."

For the investigation of indeterminate HIV-1 or HIV-2:

- "We recommend that any HIV-1 or HIV-2 serology that does not fit into a clear pattern of a confirmed laboratory diagnosis is fully investigated for the presence or absence of HIV-2 infection, and that this should be established by PCR for HIV-2 proviral DNA."

For measuring HIV-2 viral load:

- "If the pre-treatment viral load was detectable, the viral load should be measured at 1, 3 and 6 months after starting or changing ART and then 3–6 monthly.
- "If the pre-treatment viral load was undetectable, the viral load should be measured at 1 month and then 6 monthly."
- The HIV-2 viral load should be repeated in those on ART when it has been maximally suppressed and then becomes detectable."
- Testing for drug resistance should be performed in those on ART when the HIV-2 viral load has

been maximally suppressed and then becomes repeatedly detectable."

For resistance testing:

- "Resistance testing should be performed at diagnosis, prior to treatment initiation and at virological failure, if the HIV-2 viral load meets the threshold of ≥500 copies/mL" (BHIVA, 2021).

European AIDS Clinical Society (EACS)

The EACS recommends a genotypic resistance test to be ideally done at the time of HIV diagnosis; testing "should not delay ART initiation (it may be re-adjusted after genotypic test results). Resistance testing is also recommended to be performed in the setting of virological failure, "preferably on failing therapy (usually routinely available for HIV-VL levels >200-500 copies/mL and in specialized laboratories for lower levels of viremia) and obtain historical resistance testing for archived mutations." For pregnant women, the EACS recommends performing resistance testing on women whose HIV-VL is not undetectable at third trimester, and "consider changing to or adding INSTI (RAL or DTG) if not on this class to obtain rapid HIV-VL decline." When considering PEP, the EACS recommends resistance testing if the HIV-VL is detectable in an HIV-positive source person on ART. They also recommend baseline resistance testing when considering a combination regiment for ART-naïve children and adolescents living with HIV. Resistance testing should also be used to help guide the choice of treatment.

Additional genotypic recommendations include if the patient was not previously tested or if the patient is at risk of a super-infection. Genotypic resistance testing is also required prior to beginning treatment with doravirine. When switching strategies for "virologically suppressed persons," Proviral DNA genotyping may be useful in persons with multiple virological failures, unavailable resistance history or low-level viremia at the time of switch. Results ought to be taken cautiously as proviral DNA genotype may not detect previous resistance mutations and can also detect clinically irrelevant mutations. Therefore, routine proviral DNA genotyping is currently not recommended." The EACS recommends a genotypic test over a phenotypic test as genotype tests are more available and more sensitive (EACS, 2022).

The American Academy of Pediatrics (AAP)

The AAP recommends:

- "Routine HIV screening is recommended for all youth 15 years or older, at least once, in health care settings."
- "After initial screening, youth at increased risk, including sexually active youth, should be rescreened at least annually, potentially as frequently as every 3 to 6 months if at high risk (male youth reporting male sexual contact, active injection drug users, transgender youth; having sexual partners who are HIV-infected, of both genders, or injection drug users; exchanging sex for drugs or money; or those who have had a diagnosis of or request testing for other STIs)."
- "Youth who request HIV screening at any time should be tested, even in the absence of reported risk factors" (Hsu & Rakhmanina, 2022)

The Bright Futures/AAP Periodicity Schedule describes the screenings, assessments, physical examinations, procedures, and timing of anticipatory guidance recommended for each age-related visit. These guidelines provide the following recommendation for HIV screening:

- STI/HIV screening annually starting at 11 years old, with at least one HIV screening between 15 and 21 (AAP, 2023).

United States Preventive Services Task Force (USPSTF)

The USPSTF recommends screening adolescents under 15 who are at increased risk, adolescents and adults aged 15 to 65 years, and younger adolescents and older adults who are at increased risk should.

The USPSTF also recommends screening all pregnant women for HIV, including those in labor who are untested and whose HIV status is unknown (USPSTF, 2019). The CDC recognizes and supports these guidelines (CDC, 2020).

Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: http://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, please visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

The primary RT-PCR tests for HIV-1 have been approved by the FDA:

In May 2007, the FDA approved the Abbott RealTime HIV-1 Amplification Reagent Kit. From the FDA website: "The Abbott RealTime HIV-1 assay is an in vitro reverse transcription-polymerase chain reaction (RT-PCR) assay for the quantitation of Human Immunodeficiency Virus type 1 (HIV-1) on the automated m2000 System in human plasma from HIV-1 infected individuals over the range of 40 to 10,000,000 copies/mL" (FDA, 2007a).

On May 11, 2007, the FDA approved the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test. From the FDA website: "The COBAS AmpliPrep/COBAS TaqMan HIV-1 is an in vitro nucleic acid amplification test for the quantitation of Human Immunodeficiency Virus (HIV-1) nucleic acid in human plasma, using the COBAS AmpliPrep Instrument for automated sample preparation and the COBAS TaqMan Analyzer or COBAS TaqMan 48 Analyzer for automated amplification and detection. This test is intended for use in conjunction with clinical presentation and other laboratory markers of disease progress for the clinical management of HIV-1 infected patients" (FDA, 2007b).

In 2016, the FDA approved the Aptima® HIV-1 Quant Assay. From the FDA website: "The Aptima HIV-1 Quant assay is an in vitro nucleic acid amplification test (NAAT) for the quantitation of human immunodeficiency virus type 1 (HIV-1) RNA in human plasma from HIV-1 infected individuals on the fully automated Panther® system. The Aptima HIV-1 Quant assay quantitates HIV-1 RNA groups M, N, and O over the range of 30 to 10,000,000 copies/ mL." On November 20, 2020, this assay was given an FDA approval for dual use for diagnosis and viral load monitoring for HIV-1 (BusinessWire, 2020; FDA, 2020).

The following screening antibody tests are FDA-approved to differentiate HIV-1 from HIV-2:

On August 26, 2019, the FDA approved the Geenius HIV-1/2 Supplemental Assay. From the FDA Website: "The Geenius™ HIV 1/2 Supplemental Assay is a single-use immunochromatographic assay for the confirmation and differentiation of individual antibodies to Human Immunodeficiency Virus Types 1 and 2 (HIV-1 and HIV-2) in serum or plasma samples (EDTA, lithium heparin, sodium citrate, and CPD)

from blood donors. The Geenius™ HIV 1/2 Supplemental Assay is intended for use as an additional, more specific test for human serum and plasma samples with repeatedly reactive results by an FDA licensed blood donor screening test for antibodies to HIV-1/HIV-2. The results of the Geenius™ HIV 1/2 Supplemental Assay are read and interpreted only with the Geenius™ Reader with dedicated software." 200 known HIV-2 positive samples were classified by Geenius, with 77 interpreted as only HIV-2 positive, 108 with HIV-2 with HIV-1 cross reactivity, 12 as undifferentiated, and 3 as HIV-2 indeterminate (FDA, 2019).

On July 23, 2015, the FDA approved the BioPlex 2200 HIV Ag-Ab Assay. From the FDA Website: "The BioPlex 2200 HIV Ag-Ab assay is a multiplex flow immunoassay intended for the simultaneous qualitative detection and differentiation of the individual analytes HIV-1 p24 antigen, HIV-1 (groups M and O) antibodies, and HIV-2 antibodies in human serum or plasma (fresh or frozen K2 EDTA, K3 EDTA, lithium heparin, sodium heparin; fresh citrate). This assay is intended as an aid in the diagnosis of infection with HIV-1 and/or HIV-2, including acute (primary) HIV-1 infection. The assay may also be used as an aid in the diagnosis of infection with HIV-1 and/or HIV-2 in pediatric subjects as young as two years of age, and pregnant women." The test was found to differentiate all 1363 HIV-1 samples correctly and 188 of 200 HIV-2 samples correctly (with 12 "undifferentiated") (FDA, 2015).

In 2020 and 2022, the FDA approved the Alinity m HIV-1 assay as an in vitro reverse transcription-polymerase chain reaction (RT-PCR) assay for the detection and quantification of HIV-1. It is to be used both for confirmation of HIV-1 infection and for monitoring of HIV-1 infected individuals. From the FDA website: "The Alinity m HIV-1 assay is intended for use to monitor disease prognosis by measuring baseline plasma HIV-1 RNA level and to assess response to antiretroviral treatment by measuring changes in plasma HIV-1 RNA levels. Performance for quantitative monitoring is not established with serum specimens." The assay can also be used as a supplemental test to confirm HIV-1 in individuals who have "reactive results" with HIV immunoassays (FDA, 2022).

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
86689	Antibody; HTLV or HIV antibody, confirmatory test (eg, Western Blot)
86701	Antibody; HIV-1
86702	Antibody; HIV-2
86703	Antibody; HIV-1 and HIV-2, single result
87389	Infectious agent antigen detection by immunoassay technique, (eg, enzyme immunoassay [EIA], enzyme-linked immunosorbent assay [ELISA], fluorescence immunoassay [FIA], immunochemiluminometric assay [IMCA]) qualitative or semiquantitative; HIV-1 antigen(s), with HIV-1 and HIV-2 antibodies, single result
87390	Infectious agent antigen detection by immunoassay technique (eg, enzyme immunoassay [EIA], enzyme-linked immunosorbent assay [ELISA], fluorescence immunoassay [FIA], immunochemiluminometric assay [IMCA]), qualitative or semiquantitative; HIV-1
87391	Infectious agent antigen detection by immunoassay technique (eg, enzyme immunoassay [EIA], enzyme-linked immunosorbent assay [ELISA], fluorescence immunoassay [FIA], immunochemiluminometric assay [IMCA]), qualitative or semiquantitative; HIV-2

CPT	Code Description
87534	Infectious agent detection by nucleic acid (DNA or RNA); HIV-1, direct probe technique
87535	Infectious agent detection by nucleic acid (DNA or RNA); HIV-1, amplified probe technique, includes reverse transcription when performed
87536	Infectious agent detection by nucleic acid (DNA or RNA); HIV-1, quantification, includes reverse transcription when performed
87537	Infectious agent detection by nucleic acid (DNA or RNA); HIV-2, direct probe technique
87538	Infectious agent detection by nucleic acid (DNA or RNA); HIV-2, amplified probe technique, includes reverse transcription when performed
87539	Infectious agent detection by nucleic acid (DNA or RNA); HIV-2, quantification, includes reverse transcription when performed
87806	Infectious agent antigen detection by immunoassay with direct optical (ie, visual) observation; HIV-1 antigen(s), with HIV-1 and HIV-2 antibodies
87900	Infectious agent drug susceptibility phenotype prediction using regularly updated genotypic bioinformatics
87901	Infectious agent genotype analysis by nucleic acid (DNA or RNA); HIV-1, reverse transcriptase and protease regions
87903	Infectious agent phenotype analysis by nucleic acid (DNA or RNA) with drug resistance tissue culture analysis, HIV 1; first through 10 drugs tested
87904	Infectious agent phenotype analysis by nucleic acid (DNA or RNA) with drug resistance tissue culture analysis, HIV 1; each additional drug tested (List separately in addition to code for primary procedure)
87906	Infectious agent genotype analysis by nucleic acid (DNA or RNA); HIV-1, other region (eg, integrase, fusion)
0219U	Infectious agent (human immunodeficiency virus), targeted viral next-generation sequence analysis (ie, protease [PR], reverse transcriptase [RT], integrase [INT]), algorithm reported as prediction of antiviral drug susceptibility Proprietary test: Sentosa® SQ HIV-1 Genotyping Assay Lab/Manufacturer: Vela Diagnostics USA, Inc
G0432	Infectious agent antibody detection by enzyme immunoassay (EIA) technique, HIV-1 and/or HIV-2, screening
G0433	Infectious agent antibody detection by enzyme-linked immunosorbent assay (ELISA) technique, HIV-1 and/or HIV-2, screening
G0435	Infectious agent antibody detection by rapid antibody test, HIV-1 and/or HIV-2, screening
G0475	HIV antigen/antibody, combination assay, screening
S3645	HIV-1 antibody testing of oral mucosal transudate

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

Evidence-based Scientific References

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Revision History

Revision Date	Summary of Changes
03/06/2024	Reviewed and Updated: Updated the background, guidelines and recommendations, and evidence-based scientific references. Literature review did not necessitate any modifications to coverage criteria.
03/01/2023	Reviewed and Updated: Updated background, guidelines, and evidence-based scientific references. Literature review necessitated the following changes in coverage criteria: Information and coverage for HIV from G2009, G2042, M2093, and M2116 were combined into a single policy. This led to a title change from "Plasma HIV-1 and HIV-2 RNA Quantification for HIV Infection" to "Human Immunodeficiency Virus (HIV)" Added CPT 86689, 86701, 86702, 86703, 87389, 87390, 87391, 87534, 87535, 87537, 87538, 87806, 87900, 0219U
03/09/2022	Annual review: Updated background, guidelines and recommendations, and evidence-based scientific references. Literature review did not necessitate modification to coverage criteria
03/03/2021	Annual Review: Updated background, guidelines, and evidence-based scientific references. Literature review necessitated the addition of coverage for viral load testing to predict maternal-fetal transmission, per 2018 ACOG and 2020 SMFM.
03/10/2020	Annual Review: Updated background, guidelines, and evidence-based scientific references. Previous E&I CC was changed to DNMCC with a preceding statement regarding a lack of available published scientific literature. Literature review necessitated the addition of HIV-2 RNA quantification based on updated 2019 DHHS guidelines. Title changed to "Plasma HIV-1 and HIV-2 RNA Quantification for HIV Infection" to reflect the inclusion of HIV-2 testing.
03/01/2019	Annual Review: Updated background, guidelines, and evidence-based scientific references. Literature review did not necessitate any modification to the CC. Updates did not necessitate CPT coding changes.
03/16/2018	Annual review: Literature review did not necessitate any modification to coverage criteria. No changes in coding.
03/20/2017	Annual review: Literature did not necessitate any change in coverage criteria. References reviewed.
02/26/2016	Annual review: Updated content (added coverage criteria 1 and limitations) and

	references in recognition of CMS NCD released 4Q15.
11/16/2015	Initial presentation

Identification of Microorganisms using Nucleic Acid Probes

Policy Number: AHS – M2097 – Identification of Microorganisms Using Nucleic Acid Probes	Prior Policy Name and Number, as applicable:
Initial Presentation Date: 09/18/2015 Effective Date: February 1, 2025	

Policy Description

Nucleic acid hybridization technologies utilize complementary properties of the DNA double-helix structures to anneal together DNA fragments from different sources. These techniques are utilized in polymerase chain reaction (PCR) and fluorescent resonance energy transfer (FRET) techniques to identify microorganisms (Khan, 2014).

A discussion of every infectious agent that might be detected with a probe technique is beyond the scope of this policy. Many probes have been combined into panels of tests. For the purposes of this policy, only individual probes are reviewed.

For guidance on nucleic acid identification of *Candida* in vaginitis, please refer to AHS-M2057-Diagnosis of Vaginitis Including Multi-Target PCR Testing.

Related Policies

Policy Number	Policy Title
AHS-G2036	Hepatitis Testing
AHS-G2143	Lyme Disease

AHS-G2149	Pathogen Panel Testing
AHS-G2157	Diagnostic Testing of Common Sexually Transmitted Infections
AHS-G2158	Testing for Vector-Borne Infections
AHS-M2057	Diagnosis of Vaginitis

Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) The coverage status of nucleic acid identification using direct probe, amplified probe, or quantification for the microorganism's procedure codes is summarized in Table 1 below. "MCC" in the table below indicates that the test **MEETS COVERAGE CRITERIA**; while "DNMCC" tests indicates that the test **DOES NOT MEET COVERAGE CRITERIA.**

Microorganism	Direct Probe	Amplified Probe	Quantification
Bartonella henselae* or *quintana		87471 (MCC)	87472 (DNMCC)
Non-vaginal *Candida* species	87480 (DNMCC)	87481 (DNMCC)	87482 (DNMCC)
Chlamydia pneumoniae	87485 (MCC)	87486 (MCC)	87487 (DNMCC)
Clostridium difficile		87493 (MCC)	
Cytomegalovirus	87495 (MCC)	87496 (MCC)	87497 (MCC)
***Enterococcus*, Vancomycin-resistant (e.g., enterococcus vanA, vanB)**		87500 (MCC)	
Enterovirus		87498 (MCC)	
Hepatitis G	87525 (DNMCC)	87526 (DNMCC)	87527 (DNMCC)
Herpes virus-6	87531 (DNMCC)	87532 (DNMCC)	87533 (MCC)
Legionella pneumophila	87540 (MCC)	87541 (MCC)	87542 (DNMCC)
Orthopoxvirus		87593 (MCC)	
Mycoplasma pneumoniae	87580 (MCC)	87581 (MCC)	87582 (DNMCC)
Respiratory syncytial virus		87634 (MCC)	
Staphylococcus aureus		87640 (MCC)	
***Staphylococcus aureus*, methicillin resistant**		87641 (MCC)	

2) Simultaneous ordering of any combination of direct probe, amplified probe, and/or quantification for the same organism in a single encounter **DOES NOT MEET COVERAGE CRITERIA**.

Table of Terminology

Term	Definition
CDC	Centers for Disease Control and Prevention
CDI	*Clostridioides difficile* infection
CIDT	Culture-independent diagnostic test
CMV	Cytomegalovirus
CPT	Current procedural terminology

DFA	Direct fluorescent antibody testing
DNA	Deoxyribonucleic acid
EVD	Ebola virus disease
FDA	Food and Drug Administration
FRET	Fluorescent resonance energy transfer
HHV-6	Human herpesvirus 6
IDSA	Infectious Diseases Society of America
ITS	Internal transcribed region
Mpox	Monkeypox
MRSA	Methicillin-Resistant Staphylococcus Aureus
NAATs	Nucleic acid amplification tests
NGU	Nongonococcal urethritis
PCR	Polymerase chain reaction
PID	Pelvic inflammatory disease
qPCR	Quantitative polymerase chain reaction
rDNA	Recombinant deoxyribonucleic acid
RNA	Ribonucleic acid
rRT-PCR	Real-time reverse transcriptase-polymerase chain reaction
RSV	Respiratory syncytial virus infection
RT-PCR	Reverse transcriptase-polymerase chain reaction
SARS	Severe acute respiratory syndrome

Scientific Background

Nucleic acid hybridization technologies, including polymerase chain reaction (PCR), ligase- or helicase-dependent amplification, and transcription-mediated amplification, are beneficial tools for pathogen detection in blood culture and other clinical specimens due to high specificity and sensitivity (Khan, 2014). The use of nucleic acid-based methods to detect bacterial pathogens in a clinical laboratory setting offers "increased sensitivity and specificity over traditional microbiological techniques" due to its specificity, sensitivity, reduction in time, and high-throughput capability; however, "contamination potential, lack of standardization or validation for some assays, complex interpretation of results, and increased cost are possible limitations of these tests" (Mothershed & Whitney, 2006).

Guidelines and Recommendations

World Health Organization (WHO)

For detection of mpox, the WHO recommends "detection of viral DNA by polymerase chain reaction (PCR)" as the preferred laboratory test and recommends that any individual with a suspected case should be offered testing. They note that the best specimens for diagnosis are taken directly from the rash. Antigen and antibody detection may not be able to distinguish between orthopoxviruses (WHO, 2022).

2018 Infectious Diseases Society of America (IDSA)

Specific guidelines for testing of many organisms listed within the policy coverage criteria is found in the updated 2018 Infectious Diseases Society of America (IDSA) guidelines and recommendations titled, "A Guide to Utilization of the Microbiology Laboratory for Diagnosis of Infectious Diseases: 2018 Update by the Infectious Diseases Society of America and the American Society for Microbiology" (Miller et al., 2018). "This document is organized by body system, although many organisms are capable of causing disease in >1 body system. There may be a redundant mention of some organisms because of their propensity to infect multiple sites. One of the unique features of this document is its ability to assist clinicians who have specific suspicions regarding possible etiologic agents causing a specific type of disease. When the term "clinician" is used throughout the document, it also includes other licensed, advanced practice providers. Another unique feature is that in most chapters, there are targeted recommendations and precautions regarding selecting and collecting specimens for analysis for a disease process. It is very easy to access critical information about a specific body site just by consulting the table of contents. Within each chapter, there is a table describing the specimen needs regarding a variety of etiologic agents that one may suspect as causing the illness. The test methods in the tables are listed in priority order according to the recommendations of the authors and reviewers" (Miller et al., 2018).

Centers of Disease Control and Prevention (CDC)

Candida Auris (C. auris)

The CDC writes that "Molecular methods based on sequencing the D1-D2 region of the 28s rDNA or the Internal Transcribed Region (ITS) of rDNA can identify *C. auris*." The CDC further notes that various PCR methods have been developed for identifying *C. auris* (CDC, 2024d).

Chlamydia Pneumoniae (C. pneumoniae)

The CDC writes that RT-PCR is the "preferred" method of detecting an acute *C. pneumoniae* infection (CDC, 2024e).

Clostridioides difficile (C. diff)

The CDC states that there are four laboratory tests that can be used to diagnose *Clostridioides difficile* infection (CDI). "FDA-approved PCR assays are same-day tests that are highly sensitive and specific for the presence of a toxin-producing C. diff organism." The CDC does note that "molecular assays can be positive for C. diff in asymptomatic individuals and those who do not have an infection" and "when using multi-pathogen (multiplex) molecular methods, read the results with caution as the pre-test probability of *C. diff* infection might be less" (CDC, 2024b).

Cytomegalovirus (CMV)

The CDC states that "The enzyme-linked immunosorbent assay is the most common serologic test for measuring antibody to CMV." The CDC also notes that "congenital CMV infection cannot be diagnosed with antibody testing (IgG and IgM)" and recommends "the standard laboratory test for diagnosing congenital CMV infection is a PCR on saliva, with a confirmatory test on urine." (CDC, 2024f).

Mpox Virus

The CDC defines a suspect case of Mpox as a "new characteristic rash or meets one of the epidemiologic criteria and has a high clinical suspicion for mpox." A probable case is defined as "no suspicion of other

recent *Orthopoxvirus* exposure (e.g., Vaccinia virus in ACAM2000 vaccination) AND demonstration of the presence of *Orthopoxvirus* DNA by polymerase chain reaction of a clinical specimen OR *Orthopoxvirus* using immunohistochemical or electron microscopy testing methods OR Demonstration of detectable levels of anti-orthopoxvirus IgM antibody during the period of 4 to 56 days after rash onset." A confirmed case of Mpox is defined as "demonstration of the presence of Mpox virus DNA by polymerase chain reaction testing or Next-Generation sequencing of a clinical specimen OR isolation of Mpox virus in culture from a clinical specimen" (CDC, 2024k).

The CDC states that "Mpox is diagnosed using real time PCR tests" and further notes "clinicians should collect two swabs from each lesion (generally from 2-3 lesions) in case additional testing, such as clade-specific testing, is needed for these patients" (CDC, 2024l).

MRSA

The CDC remarks that "Providers can test some patients to see if they carry MRSA in their nose or on their skin. This test involves rubbing a cotton-tipped swab in the patient's nostrils or on the skin. The only way to know if MRSA is the cause of an infection is to test for the bacteria in a laboratory." The CDC further states "There are many methods laboratorians can use to test for MRSA" and lists that "Phenotypic methods recommended for the detection of MRSA include: cefoxitin broth microdilution, oxacillin broth microdilution, and cefoxitin disk diffusion testing." The CDC includes additional methods including "Nucleic acid amplification tests, such as the polymerase chain reaction (PCR), to detect the *mecA* gene, which mediates oxacillin resistance in staphylococci" but notes "*mecA* PCR tests will not detect novel resistance mechanisms or uncommon phenotypes (e.g., *mecC* or borderline-resistant oxacillin resistance)" (CDC, 2024h).

Non-Polio Enterovirus

The CDC remarks that their laboratories "routinely" perform qualitative testing for enteroviruses, parechoviruses, and uncommon picornaviruses and states that "CDC and some health departments test with molecular sequencing methods, or a real-time reverse transcription polymerase chain reaction (rRT-PCR) lab test" (CDC, 2024j).

Respiratory Syncytial Virus (RSV)

The CDC writes that "PCR tests can be used to diagnose anyone for RSV. Antigen tests are only effective when testing infants and young children" (CDC, 2024c).

Miscellaneous

The CDC does not mention the need to quantify [through PCR] *Bartonella*, *Legionella pneumophila,* or *Mycoplasma pneumoniae*. However, PCR can be performed for both *Bartonella, Legionella pneumophila,* and *Mycoplasma pneumonia*e specimen (CDC, 2024a, 2024g, 2024i). "Nucleic Acid Amplification Tests (NAATs) are the preferred method of diagnostic testing for *M. pneumoniae* infections" (CDC, 2024i). No guidance was found on Hepatitis G.

Committee on Infectious Diseases, American Academy of Pediatrics, 31st Edition (2018-2021, Red Book)

The Committee on Infectious Diseases released joint guidelines with the American Academy of Pediatrics. In it, they note that "the presumptive diagnosis of mucocutaneous candidiasis or thrush

usually can be made clinically." They also state that FISH probes may rapidly detect *Candida* species from positive blood culture samples, although PCR assays have also been developed for this purpose (AAP Committee on Infectious Diseases, 2018).

European Centre for Disease Prevention and Control (ECDC)

On May 23, 2022, the ECDC released a rapid risk assessment of the Mpoxmulti-country outbreak. They recommend that patients with probable cases should be tested with a "Mpox virus specific PCR or an orthopoxvirus specific PCR assay which is then confirmed through sequencing" (ECDC, 2022b).

On June 2, 2022, ECDC released interim advice on risk communication and community engagement during the 2022 Mpox outbreak in Europe. This is a joint report with the WHO regional office for Europe. They recommend speaking to your doctor about getting tested for Mpox if you develop a rash with a fever or feeling of discomfort or illness (ECDC, 2022a).

United Kingdom Heath Security Agency (UKHSA)

The UKHSA states that "Mpox is diagnosed by PCR test for the Mpox virus (MPXV) on a viral swab taken from one or more vesicles or ulcers." Specifically, it is recommended that healthcare workers "Take a viral swab in viral culture medium or viral transport medium (for example Virocult®) from an open sore or from the surface of a vesicle. If other wounds are present, ensure that the sample is definitely taken from a vesicle, an ulcer or a crusted vesicle. Rub the swab over the lesion and place the swab in the collection tube. If there are pharyngeal lesions, a throat swab should also be taken" (UKHSA, 2024). UKHSA also suggests that "A viral throat swab can be taken for high-risk contacts of a confirmed or highly probable case who have developed systemic symptoms but do not have a rash or lesions that can be sampled. Please note that even if the throat swab is negative, the individual must continue with monitoring and isolation as instructed by their local health protection team, and should be reassessed and sampled if further symptoms develop." Lastly, "If follow-up testing is required from a confirmed or highly probable case, either because of clinical deterioration or to inform discharge from isolation to an inpatient setting, additional samples should be taken and should include the following:

- a lesion swab and throat swab in viral transport medium
- a blood sample in an EDTA tube
- a urine sample in a universal sterile container" (UKHSA, 2024).

The UKHSA states that "Following the identification of a cluster of sexually transmitted HCID Clade I mpox in 2023, there is an increased risk of mpox HCID infection circulating unrecognized on the background of Clade II infections." They therefore recommend "All diagnostic samples from all individuals testing positive for mpox should now be subject to clade confirmation. Positive mpox samples should be sent to RIPL for clade specific testing if clade differentiation is not available through local mpox testing services" (UKHSA, 2024).

The UKHSA states that mpox DNA viruses can be detected in semen up to 11 days after acute infection, and recommends that: "Following the initial 12 weeks and up to 6 months after recovery from infection, UKHSA recommends performing MPXV PCR on semen samples (and where necessary, oropharyngeal and/or rectal swabs) if the patient:

- is undergoing fertility treatment or planning pregnancy
- is undergoing planned semen storage (for example prior to chemotherapy)
- has an immunocompromised sexual partner (including a pregnant partner)

- is concerned about transmission to sexual partner or partners for any other reason and requests a test from their clinician" (UKHSA, 2024).

HHV-6 Foundation

The human herpesvirus 6 (HHV-6) foundation also states that "a negative finding in the plasma does not rule out a localized active infection in an organ (e.g. uterus, brain, thyroid, liver). Persistent HHV-6 infections have been found in the liver, brain, lungs, heart tissue and uterus, with no trace of HHV-6 DNA in the plasma. Quantitative testing on blood and tissues is preferred because it can differentiate between the very low levels occasionally found in healthy controls and high levels found in diseased tissues" (HHV-6 Foundation, 2024).

The HHV-6 foundation states that *qualitative* PCR DNA tests on whole blood are "useless for differentiating active from latent infection" but notes that the test may be useful for differentiating between herpes virus-6A and herpes virus-6B. The HHV-6 foundation states that *quantitative* PCR DNA tests on whole blood can differentiate active from latent infection "If the viral load is >200 copies per ml or 20 copies per microgram of DNA then this is an active infection."

Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

A list of current U.S. Food and Drug Administration (FDA, 2022) approved or cleared nucleic acid-based microbial tests is available at: https://www.fda.gov/medical-devices/vitro-diagnostics/nucleic-acid-based-tests.

Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
87471	Infectious agent detection by nucleic acid (DNA or RNA); Bartonella henselae and Bartonella quintana, amplified probe technique
87472	Infectious agent detection by nucleic acid (DNA or RNA); Bartonella henselae and Bartonella quintana, quantification
87480	Infectious agent detection by nucleic acid (DNA or RNA); Candida species, direct probe technique

CPT	Code Description
87481	Infectious agent detection by nucleic acid (DNA or RNA); Candida species, amplified probe technique
87482	Infectious agent detection by nucleic acid (DNA or RNA); Candida species, quantification
87485	Infectious agent detection by nucleic acid (DNA or RNA); Chlamydia pneumoniae, direct probe technique
87486	Infectious agent detection by nucleic acid (DNA or RNA); Chlamydia pneumoniae, amplified probe technique
87487	Infectious agent detection by nucleic acid (DNA or RNA); Chlamydia pneumoniae, quantification
87493	Infectious agent detection by nucleic acid (DNA or RNA); Clostridium difficile, toxin gene(s), amplified probe technique
87495	Infectious agent detection by nucleic acid (DNA or RNA); cytomegalovirus, direct probe technique
87496	Infectious agent detection by nucleic acid (DNA or RNA); cytomegalovirus, amplified probe technique
87497	Infectious agent detection by nucleic acid (DNA or RNA); cytomegalovirus, quantification
87498	Infectious agent detection by nucleic acid (DNA or RNA); enterovirus, amplified probe technique, includes reverse transcription when performed
87500	Infectious agent detection by nucleic acid (DNA or RNA); vancomycin resistance (eg, enterococcus species van A, van B), amplified probe technique
87525	Infectious agent detection by nucleic acid (DNA or RNA); hepatitis G, direct probe technique
87526	Infectious agent detection by nucleic acid (DNA or RNA); hepatitis G, amplified probe technique
87527	Infectious agent detection by nucleic acid (DNA or RNA); hepatitis G, quantification
87531	Infectious agent detection by nucleic acid (DNA or RNA); Herpes virus-6, direct probe technique
87532	Infectious agent detection by nucleic acid (DNA or RNA); Herpes virus-6, amplified probe technique
87533	Infectious agent detection by nucleic acid (DNA or RNA); Herpes virus-6, quantification
87540	Infectious agent detection by nucleic acid (DNA or RNA); Legionella pneumophila, direct probe technique
87541	Infectious agent detection by nucleic acid (DNA or RNA); Legionella pneumophila, amplified probe technique
87542	Infectious agent detection by nucleic acid (DNA or RNA); Legionella pneumophila, quantification
87580	Infectious agent detection by nucleic acid (DNA or RNA); Mycoplasma pneumoniae, direct probe technique
87581	Infectious agent detection by nucleic acid (DNA or RNA); Mycoplasma pneumoniae, amplified probe technique
87582	Infectious agent detection by nucleic acid (DNA or RNA); Mycoplasma pneumoniae, quantification
87593	Infectious agent detection by nucleic acid (DNA or RNA); orthopoxvirus (eg, monkeypox virus, cowpox virus, vaccinia virus), amplified probe technique, each
87634	Infectious agent detection by nucleic acid (DNA or RNA); respiratory syncytial virus, amplified probe technique

CPT	Code Description
87640	Infectious agent detection by nucleic acid (DNA or RNA); Staphylococcus aureus, amplified probe technique
87641	Infectious agent detection by nucleic acid (DNA or RNA); Staphylococcus aureus, methicillin resistant, amplified probe technique

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

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Revision History

Revision Date	Summary of Changes
09/04/2024	Reviewed and Updated: Updated background, guidelines, and evidence-based scientific references. Literature review necessitated the following changes in coverage criteria: Removed Mycoplasma genitalium from this table, as management of testing for M. genitalium is now contained within G2157- Diagnostic Testing of Common Sexually Transmitted Infections Changed direct probe for HHV6 (87531) from MCC to DNMCC, as quantitative testing for HHV6 can differentiate between active and latent infection, whereas qualitative does not and direct and amplified probe coverage should match for HHV6. Removed CPT code 87563

Immune Cell Function Assay

Policy Number: AHS – G2098 – Immune Cell Function Assay	Initial Presentation Date: 09/18/2015 Effective Date: 4/1/2025

Policy Description

Immune cell function assays involve measurement of peripheral blood lymphocyte response (intracellular ATP levels, proliferation) following stimulation to assess the degree of functionality of the cell-mediated immune response (Buttgereit et al., 2000).

For guidance on procedures utilizing flow cytometry, please refer to AHS-F2019 Flow Cytometry.

Related Policies

Policy Number	Policy Title
AHS-F2019	Flow Cytometry
AHS-M2091	Transplant Rejection Testing

Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

1) For all situations, an immune cell function assay (e.g., Pleximmune™, Pleximar) **DOES NOT MEET COVERAGE CRITERIA**.

Table of Terminology

Term	Definition
AAAAI	The American Academy of Allergy, Asthma & Immunology
AASLD	American Association for the Study of Liver Diseases
ACAAI	The American College of Allergy, Asthma & Immunology
AST	The American Society of Transplantation
ATP	Adenosine triphosphate
CD3	Cluster of differentiation 3
CD4	Cluster of differentiation 4
CMI	Cell-mediated immunity
CMV	Cytomegalovirus
DOR	Diagnostic odds ratio
ELISPOT	Enzyme-linked immunosorbent spot
FDA	Food and Drug Administration
GVHD	Graft-versus-host disease
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
ICS	Intracellular cytokine staining
IGRA	Interferon-gamma release assays
ISHLT	The International Society of Heart and Lung Transplantation
ICFA	Immune cell function assay
ITx	Intestine transplant
LTx	Liver transplant
MHC	Major histocompatibility complex
NLR	Negative likelihood ratio
NPV	Negative predictive value
PLR	Positive likelihood ratio
PPV	Positive predictive value
RA	Rheumatoid arthritis
SCID	Severe combined immunodeficiency disease

Scientific Background

Primary immunodeficiencies are a group of rare disorders in which part of the body's immune system is absent or functions incorrectly. These disorders occur in as many as 1:2000 live births and are most often categorized according to a combination of mechanistic and clinical descriptive characteristics (Bonilla et al., 2015). Specific cellular immunity is mediated by T cells, and defects affecting these T cells underlie the most severe immunodeficiencies. As antibody production by B cells requires intact T cell function, most T cell defects lead to combined (cellular and humoral) immunodeficiency (Butte, 2023).

In vitro studies of T cell function measure peripheral blood T cell responses to several different types of stimuli (Bonilla, 2008):

- Mitogens (such as the plant lectins phytohemagglutinin, concanavalin A, pokeweed mitogen, anti-CD3).
- Specific antigens (such as tetanus and diphtheria toxoids or *Candida albicans* antigens).
- Allogeneic lymphocytes (i.e., mixed lymphocyte culture).

Exposure of T cells to stimulus leads to their metabolic activation and polyclonal expansion (Fernandez-Ruiz et al., 2014). Response can be measured by indicators of proliferation, ATP synthesis and release, or expansion of specific subpopulations (Butte, 2023).

The evaluation of specific immune responses is essential for diagnosis of primary immune deficiencies. Screening tests used to evaluate patients with suspected primary immune deficiencies are relatively inexpensive, performed rapidly, and reasonably sensitive and specific (Notarangelo, 2010; Oliveira & Fleisher, 2010). Abnormal screening test results indicate the need for more sophisticated tests. This stepwise approach ensures an efficient and thorough evaluation of mechanisms of immune dysfunction that underlie the clinical presentation; this process includes the narrowing of diagnostic options before using costly sophisticated tests that might be required to arrive at specific diagnoses (Bonilla et al., 2015). Abnormal T-cell counts measure T-cell mitogen responses that are absent or extremely low; this is a crucial element in the diagnosis of several primary immune deficiencies, most notably, severe combined immunodeficiency (SCID) (Picard et al., 2015). Additionally, T-cell recognition of alloantigen's is the primary and central event that leads to the cascade of events that result in rejection of a transplanted organ (Vella, 2024). Several commercial assays have been developed based on the traditional assessment of T-cell stimulation to predict or assess transplant rejection.

Proprietary Testing

The ImmuKnow assay measures the ability of CD4 T-cells to respond to mitogenic stimulation by phytohemagglutinin-L in vitro by quantifying the amount of adenosine triphosphate (ATP) produced and released from these cells following stimulation (Zhang et al., 2016). Since the CD4 lymphocytes orchestrate cell-mediated immunity responses through immunoregulatory signaling, measurement of intracellular ATP levels following CD4 activation is intended to estimate the net state of immune system in immunocompromised patients (Anglicheau et al., 2023) and one of the few well-established strategies for functional immune monitoring in solid organ transplant recipients (Sottong et al., 2000).

The Pleximmune™ blood test measures the inflammatory immune response of recipient T-cells to the donor in co-culture of lymphocytes from both sources (Ashokkumar et al., 2009; Ashokkumar et al., 2017; Sindhi et al., 2016). The Pleximmune test sensitivity and specificity for predicting acute cellular rejection was found to be 84% and 81%, respectively, in a training set–validation set testing of 214 children. Early clinical experience shows that test predictions are particularly useful in planning immunosuppression in the setting of indeterminate biopsy findings or in modifying protocol-mandated treatment when combined with all other available clinical information about an individual patient (Sindhi et al., 2016).

The iQue® Immune Cell Function Assay identifies immune cells based on cell surface markers or secreted soluble mediators. This assay quantifies cytokines, adhesion molecules, enzymes, and growth factors receptors and measures cell phenotypes, cell function markers, cell viability, cell count, proliferation, and secreted effector cytokines in a single well. The iQue® assay can be used to characterize T cells and

measure various populations including memory T cells, cytotoxic T cells, and natural killer cells (Intellicyt, 2024).

Clinical Utility and Validity

A population-based study comparing the assay results in healthy controls and solid organ transplant recipients established three categories to define patient's cell-mediated immune response: strong (≥525 ng ml^{-1}), moderate (226–524 ng ml^{-1}) and low (≤225 ng ml^{-1}) (Fernandez-Ruiz et al., 2014; Kowalski et al., 2006). Numerous authors have analyzed the predictive value of the ImmuKnow® (Viracor) assay for acute rejection, as recently summarized in a meta-analysis that found a relatively high specificity (0.75) but a low sensitivity (0.43), with significant heterogeneity across studies (Fernandez-Ruiz et al., 2014; Ling et al., 2012). The ImmuKnow® assay has been examined in clinical trials for its potential use in monitoring immunosuppression medication regimens in solid organ transplant patients.

Kowalski et al. (2006) performed a meta-analysis of 504 solid organ transplant recipients (heart, kidney, kidney-pancreas, liver, and small bowel) from 10 U.S. centers. The authors found that "A recipient with an immune response value of 25 ng/ml adenosine triphosphate (ATP) was 12 times more likely to develop an infection than a recipient with a stronger immune response. Similarly, a recipient with an immune response of 700 ng/ml ATP was 30 times more likely to develop a cellular rejection than a recipient with a lower immune response value" (Kowalski et al., 2006). The authors also hypothesized an "immunological target of immune function," created by the intersection of odds ratio curves at 280 ng/ml ATP. The authors concluded "the Cylex ImmuKnow assay has a high negative predictive value and provides a target immunological response zone for minimizing risk and managing patients to stability" (Kowalski et al., 2006).

Wang et al. (2014) performed a meta-analysis of six studies which found "The pooled sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), and diagnostic odds ratio (DOR) of ImmuKnow for predicting the risk of infection were 0.51, 0.75, 1.97, 0.67, and 3.56, respectively. A DOR of 13.81, with a sensitivity of 0.51, a specificity of 0.90, a PLR of 4.45, and an NLR of 0.35, was found in the analysis of the predictive value for acute rejection." The authors concluded, "Our analysis did not support the use of the ImmuKnow assay to predict or monitor the risks of infection and acute rejection in renal transplant recipients. Further studies are needed to confirm the relationships between the ImmuKnow assay and infection and acute rejection in kidney transplantation" (Wang et al., 2014).

Jo et al. (2015) analyzed CD4 T-lymphocytes ATP levels along with lymphocyte subsets in 160 samples from 111 post-allogeneic hematopoietic stem cell transplantation (alloHSCT) patients. In patients with stable status, the six-month post-alloHSCT ImmuKnow® levels were found to be significantly higher than those tested within six months post-alloHSCT. ImmuKnow® results six months post-alloHSCT showed low positive correlation with natural killer cell count ($r = 0.328$) and the values tested later than six months post-alloHSCT were positively correlated with CD4 T cell count ($r = 0.425$).
However, ImmuKnow® levels for acute graft-versus-host disease (GVHD) or infection episodes were not significantly different compared to those for stable alloHSCT. The authors concluded that "the combined test of ImmuKnow levels and lymphocyte subsets may be helpful for immune monitoring following alloHSCT."

Ravaioli et al. (2015) aimed to "assess the clinical benefits of adjusting immunosuppressive therapy in liver recipients based on immune function assay results." A total of 100 patients received serial immune function testing via the ImmuKnow in vitro diagnostic assay (compared to 102 controls who received standard practice). The authors found that "based on immune function values, tacrolimus doses were

reduced 25% when values were less than 130 ng/mL adenosine triphosphate (low immune cell response) and increased 25% when values were greater than 450 ng/mL adenosine triphosphate (strong immune cell response)" (Ravaioli et al., 2015). The authors also found that survival and infection rates were better in the treatment arm compared to the control arm. Overall, the investigators concluded "Immune function testing provided additional data which helped optimize immunosuppression and improve patient outcomes" (Ravaioli et al., 2015).

Piloni et al. (2016) evaluated 61 lung recipients who underwent follow-up for lung transplantation between 2010 and 2014 in order to correlate ImmuKnow® values with functional immunity in lung transplant recipients. The authors found that 71 out of 127 samples (56%) showed an over-immunosuppression with an ImmuKnow® assay mean level of 112.92 ng/ml (SD ± 58.2) vs. 406.14 ng/ml (SD ± 167.7) of the rest of our cohort. In the over-immunosuppression group, the authors found 51 episodes of infection (71%). The mean absolute ATP level was significantly different between patients with or without infection (202.38 ± 139.06 ng/ml vs. 315.51 ± 221.60 ng/ml). The authors concluded that "the ImmuKnow assay levels were significantly lower in infected lung transplant recipients compared with non-infected recipients and in RAS patients" (Piloni et al., 2016).

Chiereghin et al. (2017) evaluated symptomatic infectious episodes that occurred during the first year after an organ transplant. A total of 135 infectious episodes were studied with 77 of the infections bacterial, 45 viral, and 13 fungal. Significantly lower median ImmuKnow® intracellular ATP levels were identified in patients with bacterial or fungal infections compared to infection-free patients, whereas patients with viral infection did not have a significantly different median ATP level compared to non-infected patients. The authors concluded that bacteria were responsible for most symptomatic infections post-transplant and that ImmuKnow measurements may be useful for "identifying patients at high risk of developing infection, particularly of fungal and bacterial etiology" (Chiereghin et al., 2017).

Liu et al. (2019) studied the potential of the ImmuKnow assay to diagnose infection in pediatric patients who have received a living-donor liver transplant. A total of 66 patients participated in this study and were divided into infection (n=28) and non-infection (n=38) groups. The researchers report that the "CD4+ T lymphocyte ATP value of the infection group was significantly lower compared with that of the non-infection group" (Liu et al., 2019). This suggests that for pediatric patients who have received a living-donor liver transplant, low CD4+ T lymphocyte ATP levels may be related to infection rates. The ImmuKnow assay may be a helpful tool in this scenario to predict infection.

Weston et al. (2020) used the ImmuKnow assay to adjust immunosuppression in heart transplant recipients with severe systemic infections. In particular, if a patient developed an infection, the ImmuKnow assay was used to recommend adjustments in immunosuppression. This assay was used on 80 patients; thirteen of these patients developed a more serious infection. The researchers conclude that "Heart transplant recipients with severe systemic infections presented with a decreased ImmuKnow®, suggesting over immunosuppression. ImmuKnow® can be used as an objective measurement in withdrawing immunosuppression in heart transplant recipients with severe systemic infections" (Weston et al., 2020).

Ashokkumar et al. (2017) evaluated PlexImmune through the assessment of CD-154 T-cytotoxic memory cells. A total of 280 samples (158 training set, 122 validation) from 214 children were examined. Recipient CD-154 cells induced by stimulation with donor cells were expressed as a fraction of those induced by human leukocyte antigen (HLA) nonidentical cells, and a resulting immunoreactivity index (IR) ≥1 implied increased rejection-risk. The authors found that "an IR of 1.1 or greater in posttransplant training samples and IR of 1.23 or greater in pretransplant training samples predicted liver transplant

(LTx) or intestine transplant (ITx) rejection with sensitivity, specificity, positive, and negative predictive values of 84%, 80%, 64%, and 92%, respectively, and 57%, 89%, 78%, and 74%, respectively" (Ashokkumar et al., 2017). The authors concluded that "Allospecific CD154+T-cytotoxic memory cells predict acute cellular rejection after LTx or ITx in children. Adjunctive use can enhance clinical outcomes" (Ashokkumar et al., 2017).

However, at the present time, there is no consensus on the utility of these tests, despite the amount of literature devoted to determine its real value for predicting post-transplant complications (Clark & Cotler, 2024; Fernandez-Ruiz et al., 2014; Kowalski et al., 2006; Ling et al., 2012; Rodrigo et al., 2012).

Monforte et al. (2021) studied the prognostic value of ImmuKnow® for predicting non-cytomegalovirus (CMV) infections in lung transplant patients. After their lung transplants, 92 patients were followed for six to twelve months and the assay was carried out at 6, 8, 10, and 12 months. Twenty five percent of the patients developed non-CMV infections between 6-12 months after the transplant. At six months, 15.2% of patients had a moderate immune response and 84.8% of patients had a low immune response to the infection. In the following six months, only one of the patients with a moderate immune response developed a non-CMV infection compared to the 28.2% of low immune response patients who developed a non-CMV infection. The ImmuKnow® assay had a sensitivity of 95.7%, specificity of 18.8%, positive predictive value (PPV) of 28.2%, and negative predictive value (NPV) of 92.9% in detecting a non-CMV infection. The authors conclude that "although ImmuKnow® does not seem useful to predict non-CMV infection, it could identify patients with a very low risk and help us define a target for an optimal immunosuppression" (Monforte et al., 2021).

In an open-label prospective cohort study, Xue et al. (2021) studied the use of the Cylex immune cell function assay for diagnosis of infection after liver transplant in pediatric patients. A total of 216 infants with liver transplants were followed and Cylex ATP values were measured before and after the liver transplant at weeks 1, 2, 3, 4, 8, 12 and 24. After surgery, 74.1% of the transplant patients had a diagnosed infection, 20.4% were clinically stable, and 5.6% experienced acute rejection. The median Cylex ATP value in infant PLTs post-surgery reduced significantly in the infection group compared to stable group. ROC curve analysis determined that the cut-off value of Cylex ATP was 152 ng/mL for diagnosis of infection. The authors conclude "In this study, we demonstrated that low Cylex ATP represented partly over-immunosuppression and had diagnostic value in infant PLTs with infections, which might assist individualized immunosuppression in PLT patients" (Xue et al., 2021).

Maidman et al. (2022) performed a retrospective observational study on patients from 2018 to 2020 who underwent orthotopic cardiac transplantation in a single center to investigate the predictive value of pre-transplant ImmuKnow results on rejection. When separating the patients into cohorts of low activity and moderate-high activity with the test results, they found that in the no patients experienced early organ rejection in the low pre-transplant ImmuKnow group, but 24.2% of patients experienced early rejection in the high pre-transplant ImmuKnow group with statistical significance. The researchers ultimately concluded a potential utility of utilizing pre-transplant ImmuKnow results to predict possible risk of early heart transplant rejection, and thus promote earlier intervention and immunosuppression when appropriate (Maidman et al., 2022).

Chen et al. (2023) performed a retrospective analysis of ICFA and CD3 lymphocyte counts and the connection of these counts with adverse effects after orthotopic heart transplant. A total of 381 ICFA and 493 CD3 values from the lab were obtained in 78 individuals who were six months post-surgery. Of these individuals, fourteen patients had to be treated for acute transplant rejection (evidenced through biopsy) and four patients had a ISHLT grade 2R/3A rejection. " In patients with rejection versus those

without, CD3 and ICFA values were 122 (IQR 74.5-308) cells/mm^2 and 224.5 (IQR 132-343.5) ng/ml compared to 231.8 (IQR 68-421) cells/m^2 and 191 (IQR 81.5-333) ng/mL (p = NS for both)." In conclusion, the authors found no association between the immune markers profiled and adverse outcomes but noted that there was an absence of larger pediatric studies showing that these tests were accurate and clinical useful in identifying elevated risk profiles after orthotopic health transplant; they did not recommend the routine use of these tests (Chen et al., 2023).

Guidelines and Recommendations

The American Academy of Allergy, Asthma & Immunology (AAAAI) and the American College of Allergy, Asthma & Immunology (ACAAI)

The American Academy of Allergy, Asthma & Immunology (AAAAI) and the American College of Allergy, Asthma & Immunology (ACAAI) published practice parameters for the diagnosis and management of primary immunodeficiency (Bonilla et al., 2015) which stated that:

"Evaluation of specific immune responses is essential for diagnosis of PIDDs [primary immunodeficiency diseases]. Measurement of serum immunoglobulin levels and lymphocyte responses to mitogens are useful indicators of global B- and T-cell development and function."

The guideline also lists "In vitro proliferative response to mitogens and antigens" as an advanced test used when "Abnormal screening test results indicate the need for more sophisticated tests" (Bonilla et al., 2015). The screening test indicated is flow cytometry to enumerate CD4 and CD8 T cells and NK cells.

Normal or abnormal T cell response to mitogen stimulation is listed in the diagnostic algorithm for the diagnosis of combined or syndromic immunodeficiencies. Specifically, it states that "Infants with low TREC counts should have secondary screening by using flow cytometry to enumerate T-cell numbers and the proportion of naive cells. T-cell counts of less than 1500/mm^3 or a proportion of naive cells of less than 50% should be followed up measuring the in vitro response to a mitogen, such as PHA." It is also listed as a characteristic laboratory finding for WAS, AT related disorders, Good syndrome, XLP1, MSMD, MyD88, WHIM, EV and in the management of DGS, and immuno-osseous dysplasia.

The International Society of Heart and Lung Transplantation (ISHLT)

In their recommendations for non-invasive monitoring of acute heart transplant rejection, the ISHLT made a new Class III recommendation that "use of the immune cell function assay (ImmuKnow) cannot be recommended in adult and pediatric heart transplant recipients for rejection monitoring" with a B Level of Evidence (Velleca et al., 2022).

An ISHLT consensus document for the management of antibodies in a heart transplantation was published in 2018. This document does not mention the ImmuKnow or Pleximmune assays, but does state that "Solid-phase assays, such as the Luminex SAB assay, are recommended to detect circulating antibodies" (Kobashigawa et al., 2018).

An ISHLT consensus document for the antibody-mediated rejection of the lung was published in 2016. This consensus document does not mention the ImmuKnow or Pleximmune assays (Levine et al., 2016).

The American Society of Transplantation (AST)

The American Society of Transplantation does not include the use of the ImmuKnow assay in its publication: "Recommendations for Screening, Monitoring and Reporting of Infectious Complications in Immunosuppression Trials in Recipients of Organ Transplantation" (Humar & Michaels, 2006).

Educational guidelines for the management of kidney transplant recipients in the community setting and for infectious diseases in transplant recipients published in 2009 by the American Society of Transplantation (AST) also do not include ImmuKnow® (AST, 2009).

In a 2019 update, the AST addresses immune monitoring for CMV during transplant: "Immune monitoring to measure nonspecific and CMV-specific T-cell quantity and/or function is emerging as a clinical tool to assist in CMV risk stratification and management after solid organ transplantation. Nonspecific measures such as absolute lymphocyte count, CD4+ T-cell count, and nonspecific (mitogen) T-cell immune responses have been correlated with the risk of CMV disease after solid organ transplantation. In addition, several platforms are available to assess CMV-specific T-cell responses, including interferon-gamma release assays (IGRA), enzyme-linked immunosorbent spot (ELISPOT) assays, intracellular cytokine staining (ICS) for interferon-gamma (or other cytokines) using flow cytometry, and major histocompatibility complex (MHC)-multimer-based assays that directly stain peptide-specific T-cells. Numerous studies, often single-center and observational, have highlighted the potential role of immune assays in CMV risk assessment. In general, regardless of the assay that is used, the absence of adequate CMV-specific CD4+ and/or CD8+ T-cell immunity correlates with a higher risk of CMV disease, treatment failure, and CMV relapse"(Razonable & Humar, 2019).

Third International Consensus Guidelines on the Management of Cytomegalovirus in Solid-organ Transplantation

The International Cytomegalovirus CMV Consensus Group of the Transplantation Society published an international consensus statement on the management of CMV in solid organ transplant in 2018. In it, they note that "Clinical utility studies demonstrate that alteration of patient management based on the results of an immune-based assay is feasible, safe, and cost-effective" (Kotton et al., 2018).

Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

ImmuKnow® (Viracor, previously, Cylex) is an immune cell function assay cleared for marketing by the U.S. Food and Drug Administration (FDA) in April 2002 to detect cell-mediated immunity (CMI) in an immunosuppressed patient population. Cylex obtained 510(k) clearances from the FDA to market the Immune Cell Function Assay based on substantial equivalence to two flow cytometry reagents. The FDA-indicated use of the Cylex Immune Cell Function Assay is for the detection of cell-mediated immunity in an immunosuppressed population. A subsequent 510(k) marketing clearance for a device modification was issued by the FDA for this assay in 2010. There were no changes to the indications or intended use.

In August 2014, Pleximmune™ (Plexision, Pittsburgh, PA) was approved by FDA through the humanitarian device exemption process. The test is intended for use in the pre-transplantation and early and late post-transplantation period in pediatric liver and small bowel transplant patients for the purpose of predicting the risk of transplant rejection within 60 days after transplantation or 60 days after sampling.

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
81560	Transplantation medicine (allograft rejection, pediatric liver and small bowel), measurement of donor and third-party-induced CD154+T-cytotoxic memory cells, utilizing whole peripheral blood, algorithm reported as a rejection risk score Proprietary test: Pleximmune™ Lab/Manufacturer: Plexision, Inc
86352	Cellular function assay involving stimulation (eg, mitogen or antigen) and detection of biomarker (eg, ATP)
0018M	Transplantation medicine (allograft rejection, renal), measurement of donor and third-party-induced CD154+T-cytotoxic memory cells, utilizing whole peripheral blood, algorithm reported as a rejection risk score Proprietary test: Pleximark Lab/Manufacturer: Plexision, Inc

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

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Revision History

Revision Date	Summary of Changes
12/04/2024	Reviewed and Updated: Updated the background, guidelines and recommendations, and evidence-based scientific references. Literature review did not necessitate any modifications to coverage criteria.

Immunohistochemistry

Policy Number: AHS –P2018 – Immunohistochemistry	Initial Presentation Date: 11/15/2015 Effective Date: 4/1/2025

Policy Description

Immunohistochemistry (IHC) is a very sensitive and specific staining technique that uses anatomical, biochemical, and immunological methods to identify cells, tissues, and organisms by the interaction of target antigens with highly specific monoclonal antibodies and visualization though the use of a biochemical tag or label (Fitzgibbons et al., 2014).

Related Policies

Policy Number	Policy Title
N/A	Not Applicable

Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) Code 88342 should be used for the first single antibody procedure and is reimbursed at one unit per specimen, up to four specimens, per date of service.

2) Code 88341 should be used for each additional single antibody per specimen and is reimbursed up to a maximum of 13 units per date of service.

3) Code 88344 should be used for each multiplex antibody per specimen, up to six specimens, per date of service.

Table of Terminology

Term	Definition
AFP	Alpha-fetoprotein
ARID1A	AT-rich interactive domain-containing protein 1A
ASCO	The American Society of Clinical Oncology
Bcl2	*BCL2 apoptosis regulator*
b-HCG	Beta human chorionic gonadotropin
BRCA1	Breast cancer type 1 susceptibility protein gene
BAP1	*BRCA1* associated protein 1
CAIX	Carbonic anhydrase IX
CAP	College of American Pathologists
CD1a	Cluster of differentiation 1a
CD5	Cluster of differentiation 5
CD10	Cluster of differentiation 10
CD21	Cluster of differentiation 21
CD30	Cluster of differentiation 30
CD31	Cluster of differentiation 31
CD34	Cluster of differentiation 34
CD35	Cluster of differentiation 35
CD43	Cluster of differentiation 43
CD56	Cluster of differentiation 56
CD99	Cluster of differentiation 99
CD117	Cluster of differentiation 117
CDH17	*Cadherin-17*
CDK4	*Cyclin-dependent kinase 4*
CDX2	Caudal-type homeobox 2
CEA	Carcinoembryonic antigen
CK	Creatine kinase
CK17	Cytokeratin 17
CK20	Cytokeratin 20
CK5/6	Cytokeratin 5/6
CK903	Cytokeratin 903
CLIA'88	Clinical Laboratory Improvement Amendments of 1988
CMS	Centers for Medicare and Medicaid Services
CRC	Colorectal cancer
D2-40	Anti-Podoplanin
DNA	Deoxyribonucleic acid
DOG1	Delay of germination 1
ERG	ETS-related gene
ESMO	The European Society of Medical Oncology

FDA	Food and Drug Administration
FISH	Fluorescence in situ hybridization
Fli-1	Friend leukemia integration 1
FOXL2	Forkhead box protein L2
GATA3	GATA binding protein 3
GCDFP15	Gross cystic disease fluid protein 15
GI	Gastrointestinal tract
HepPar-1	General hepatocyte paraffin 1
HER2	*Human epidermal growth factor receptor 2*
HMB-45	Human melanoma black-45
HNF-1b	Hepatocyte nuclear factor 1 beta
HPV	Human papillomavirus
IHC	Immunohistochemistry
IMP3	U3 small nucleolar ribonucleoprotein protein IMP3
INI1	Integrase interactor 1
ISH	In situ hybridization
KIM-1	Kidney injury molecule-1
LDTs	Laboratory-developed tests
Maspin	Mammary serine protease inhibitor
MCPyV	Merkel cell polyomavirus
MDM2	Mouse double minute 2 homolog
MIB-1	MIB E3 ubiquitin protein ligase 1
mIHC	Multiplex immunohistochemistry
MiTF	Microphthalmia-associated transcription factor
MLH1	MutL homolog 1
MMR	Mismatch repair protein
MPO	Myeloperoxidase
MSA	Mammary serum antigen
MSH2	Mismatch repair protein Msh2
MSI	Microsatellite instability
MUC4	Mucin 4
MUC5AC	Mucin 5AC
MyoD1	Myoblast determination protein 1
NANOG	*Nanog Homeobox*
napsin A	Novel aspartic proteinase of the pepsin family A
NCCN	The National Cancer Coalition Network
NKX2.2	Homeobox protein
NKX3.1	Homeobox protein
NY-ESO-1	New York esophageal squamous cell carcinoma 1
OCT4	Octamer-binding transcription factor 4
p16	Cyclin-dependent kinase inhibitor 2A
p40	Protein subunit
P504S	Cytoplasmic protein

p63	Tumor protein p63
pan-Trk	Pan-tropomyosin-related-kinase
PAX2	Paired box 2
PAX8	Paired box 8
PDX1	Insulin promoter factor 1
PNET	Primitive neuro-ectodermal tumor
PSA	Prostate-specific antigen
PSAP	Phosphoserine aminotransferase
PTEN	Phosphatase and tensin homolog
pVHL	Von hippel–lindau tumor suppressor
RB	Retinoblastoma protein
RCC	Renal cell carcinoma
RCCma	Renal cell carcinoma marker
S100P	S100 calcium-binding protein p
SALL4	Sal-like protein 4
SATB2	Special AT-rich sequence-binding protein 2
SF-1	Steroidogenic factor 1
SOX10	SRY-box transcription factor 10
TFE3	Transcription factor E3
TLE1	Transducin-like enhancer protein 1
TTF1	Transcription termination factor, RNA polymerase I
UPII	Uroplakin II
WT1	Wilms tumor protein

Scientific Background

Immunohistochemistry (IHC) is used to identify certain components of tissues or cells (also known as immunocytochemistry) via use of specific antibodies that can be visualized through a staining technique. The premise behind IHC is that distinct tissues and cells contain a unique set of antigens that allows them to be identified and differentiated. The selection of antibodies used for the evaluation of a specimen varies by the source of the specimen, the question to be answered, and the pathologist performing the test.

Importantly, an entirely sensitive and specific IHC marker rarely exists, and therefore, determinations are typically based on a pattern of positive and negative stains for a panel of several antibodies. The four most common IHC staining patterns include nuclear staining, cytoplasmic staining, membrane staining, and extracellular staining (Tuffaha et al., 2018). A single IHC marker approach (other than for pathogens such as cytomegalovirus or BK virus) is strongly discouraged since aberrant expression of a highly specific IHC marker can rarely occur. However, aberrant expression of the entire panel of highly specific IHC markers is nearly statistically impossible (Lin & Chen, 2014).

Multiplex immunohistochemistry (mIHC) is a particular IHC technique that allows multiple targets in a single tissue to be detected simultaneously; this approach is able to characterize "the tumor microenvironment including vascular architecture and hypoxia, cellular proliferation, cell death as well as drug distribution" (Kalra & Baker, 2017). Hence, mIHC can assist in the development of parameter tumor maps. Other researchers have utilized mIHC for its novel ability to provide quantitative data on different

types of tumor-infiltrating immune cells within a single tissue; this may improve cancer patient immunotherapy stratification (Hofman et al., 2019).

Clinical Utility and Validity

Immunohistochemistry can be used for a variety of purposes including: differentiation of benign from malignant tissue, differentiation among several types of cancer, selection of therapy, identification of the origin of a metastatic cancer, and identification of infectious organisms (Shah et al., 2012). IHC has many uses in the realm of tumor identification, and it has even been clinically used to pinpoint various breast cancer-specific markers, such as progesterone and estrogen receptors, gross cystic duct fluid protein, and mammaglobin (Hainsworth & Greco, 2023). Further, overexpression of the *HER2* oncogene, a predicative breast cancer biomarker, is often identified via IHC (Yamauchi & Bleiweiss, 2023). In regards to tumor identification, a specific type of IHC, known as pan-Trk IHC, has been shown to positively identify inflammatory myofibroblastic tumors with a nuclear and cytoplasmic staining pattern that may assist in targeted therapy (Yamamoto et al., 2019).

Antibodies for use in IHC are available as single antibody reagents or in mixtures of a combination of antibodies. More than 200 diagnostic antibodies are generally available in a large clinical IHC laboratory, and hundreds of antibodies are usually available in research laboratories. The list of new antibodies is growing rapidly with the discovery of new biomarkers by molecular methodologies (Lizotte et al., 2016). Several studies have shown that a relatively low number of antibodies are capable of accurately diagnosing specific cancers and identifying the primary source of a metastasis (Le Stang et al., 2019; Lizotte et al., 2016; Prok & Prayson, 2006).

Common markers to identify tumor origin (Lin & Chen, 2014):

Primary Site	**Markers**
Lung adenocarcinoma	TTF1, napsin A
Breast carcinoma	GATA3, ER, GCDFP15
Urothelial carcinoma	GATA3, UPII, S100P, CK903, p63
Squamous cell carcinoma	p40, CK5/6
RCC, clear cell type	PAX8, RCCma, pVHL, KIM-1
Papillary RCC	P504S, RCCma, pVHL, PAX8, KIM-1
Translocational RCC	TFE3
Hepatocellular carcinoma	Arginase-1, glypican-3, HepPar-1
Adrenal cortical neoplasm	Mart-1, inhibin-a, calretinin, SF-1
Melanoma	S100, Mart-1, HMB-45, MiTF, SOX10
Merkel cell carcinoma	CK20 (perinuclear dot staining), MCPyV
Mesothelial origin	Calretinin, WT1, D2-40, CK5/6, mesothelin
Neuroendocrine origin	Chromogranin, synaptophysin, CD56
Upper GI tract	CDH17, CDX2, CK20

Lower GI tract	CDH17, SATB2, CDX2, CK20
Intrahepatic cholangiocarcinoma	pVHL, CAIX
Pancreas, acinar cell carcinoma	Glypican-3, antitrypsin
Pancreas, ductal adenocarcinoma	MUC5AC, CK17, Maspin, S100P, IMP3
Pancreas, neuroendocrine tumor	PR, PAX8, PDX1, CDH17, islet-1
Pancreas, solid pseudopapillary tumor	Nuclear b-catenin, loss of Ecadherin, PR, CD10, vimentin
Prostate, adenocarcinoma	PSA, NKX3.1, PSAP, ERG
Ovarian serous carcinoma	PAX8, ER, WT1
Ovarian clear cell carcinoma	pVHL, HNF-1b, KIM-1, PAX8
Endometrial stromal sarcoma	CD10, ER
Endometrial adenocarcinoma	PAX8/PAX2, ER, vimentin
Endocervical adenocarcinoma	PAX8, p16, CEA, HPV in situ hybridization, loss of PAX2
Thyroid follicular cell origin	TTF1, PAX8, thyroglobulin
Thyroid medullary carcinoma	Calcitonin, TTF1, CEA
Hyalinizing trabecular adenoma of the thyroid	MIB-1 (unique membranous staining pattern)
Salivary duct carcinoma	GATA3, AR, GCDFP-15, HER2/neu
Thymic origin	PAX8, p63, CD5
Seminoma	SALL4, OCT4, CD117, D2-40
Yolk sac tumor	SALL4, glypican-3, AFP
Embryonal carcinoma	SALL4, OCT4, NANOG, CD30
Choriocarcinoma	b-HCG, CD10, SALL4
Sex cord–stromal tumors	SF-1, inhibin-a, calretinin, FOXL2
Vascular tumor	ERG, CD31, CD34, Fli-1
Synovial sarcoma	TLE1, cytokeratin
Chordoma	Cytokeratin, S100
Desmoplastic small round cell tumor	Cytokeratin, CD99, desmin, WT1 (N-terminus)
Alveolar soft part sarcoma	TFE3
Rhabdomyosarcoma	Myogenin, desmin, MyoD1
Smooth muscle tumor	SMA, MSA, desmin, calponin
Ewing sarcoma/PNET	NKX2.2, CD99, Fli-1
Myxoid and round cell liposarcoma	NY-ESO-1

Low-grade fibromyxoid sarcoma	MUC4
Epithelioid sarcoma	Loss of INI1, CD34, CK
Atypical lipomatous tumor	MDM2 (MDM2 by FISH is a more sensitive and specific test), CDK4
Histiocytosis X	CD1a, S100
Angiomyolipoma	HMB-45, SMA
Gastrointestinal stromal tumor	CD117, DOG1
Solitary fibrous tumor	CD34, Bcl2, CD99
Myoepithelial carcinoma	Cytokeratin and myoepithelial markers; may lose INI1
Myeloid sarcoma	CD43, CD34, MPO
Follicular dendritic cell tumor	CD21, CD35
Mast cell tumor	CD117, tryptase

Guidelines and Recommendations

Guidelines are lacking regarding the selection and number of antibodies that should be used for most immunohistochemistry evaluations. However, IHC is broadly used for conditions such as cancers, which are mentioned across many different societies. The below section is not a comprehensive list of guidance for immunohistochemistry.

College of American Pathologists (CAP)

The College of American Pathologists has published several reviews in Archives of Pathology & Laboratory Medicine that detail the quality control measures for IHC; further, CAP has also published more than 100 small IHC panels to address the frequently asked questions in diagnosis and differential diagnosis of specific entities. These diagnostic panels are based on literature, IHC data, and personal experience. A single IHC marker approach (other than for pathogens such as cytomegalovirus or BK virus) is strongly discouraged since aberrant expression of a highly specific IHC marker can rarely occur. However, aberrant expression of the entire panel of highly specific IHC markers is nearly statistically impossible (Lin & Chen, 2014; Lin & Liu, 2014).

In 2024, CAP published an update to their guidelines on the principles of analytic validation of immunohistochemical assays. The guidelines include the following recommendations (Goldsmith et al., 2024):

1. "Laboratories must analytically validate all laboratory developed IHC assays and verify all FDA-cleared IHC assays before reporting results on patient tissues.
2. For initial analytic validation or verification of every assay used clinically, laboratories should achieve at least 90% overall concordance between the new assay and the comparator assay or expected results.
3. For initial analytic validation of nonpredictive laboratory-developed assays, laboratories should test a minimum of 10 positive and 10 negative tissues. When the laboratory medical director

determines that fewer than 20 validation cases are sufficient for a specific marker (eg, rare antigen), the rationale for that decision needs to be documented.

4. For initial analytic validation of all laboratory-developed predictive marker assays, laboratories should test a minimum of 20 positive and 20 negative tissues. When the laboratory medical director determines that fewer than 40 validation tissues are sufficient for a specific marker, the rationale for that decision needs to be documented.
5. For initial analytic verification of all unmodified FDA-approved predictive marker assays, laboratories should follow the specific instructions provided by the manufacturer. If the package insert does not delineate specific instructions for assay verification, the laboratory should test a minimum of 20 positive and 20 negative tissues. When the laboratory medical director determines that fewer than 40 verification tissues are sufficient for a specific marker, the rationale for that decision needs to be documented.
6. For initial analytic validation of laboratory-developed assays and verification of FDA-approved or cleared predictive immunohistochemical assays with distinct scoring schemes (eg, HER2, PD-L1), laboratories should separately validate or verify each assay-scoring system combination with a minimum of 20 positive and 20 negative tissues. The set should include challenges based on the intended clinical use of the assay.
7. For laboratory-developed assays with both predictive and nonpredictive applications using the same scoring criteria, laboratories should treat these assays as predictive markers and test a minimum of 20 positive and 20 negative cases.
8. Laboratories should use validation tissues that have been processed using the same fixative and processing methods as cases that will be tested clinically, when possible.
9. For analytic validation of IHC performed on cytologic specimens that are not fixed in the same manner as the tissues used for initial assay validation, laboratories should perform separate validations for every new analyte and corresponding fixation method before placing them into clinical service.
10. A minimum of 10 positive and 10 negative cases is recommended for each validation performed on cytologic specimens, if possible. The laboratory medical director should consider increasing the number of cases if predictive markers are being validated. If the minimum of 10 positive and 10 negative cases is not feasible, the rationale for using fewer cases should be documented.
11. If IHC is regularly done on decalcified tissues, laboratories should test a sufficient number of such tissues to ensure that assays consistently achieve expected results. The laboratory medical director is responsible for determining the number of positive and negative tissues and the number of predictive and nonpredictive markers to test.
12. Laboratories should confirm assay performance with at least 1 known positive and 1 known negative tissue when a new antibody lot is placed into clinical service for an existing validated assay (a control tissue with known positive and negative cells is sufficient for this purpose).
13. Laboratories should confirm assay performance with at least 2 known positive and 2 known negative tissues when an existing validated assay has changed in any one of the following ways: 1. Antibody dilution 2. Antibody vendor (same clone) 3. Incubation or retrieval times (same method).
14. Laboratories should confirm assay performance by testing a sufficient number of tissues to ensure that assays consistently achieve expected results when any of the following have changed: 1. Fixative type 2. Antigen retrieval method (eg, change in pH, different buffer, different heat platform) 3. Detection system 4. Tissue processing equipment 5. Automated testing platform 6. Environmental conditions of testing (eg, laboratory relocation, laboratory water supply) The laboratory medical director is responsible for determining how many predictive and nonpredictive markers and how many positive and negative tissues to test.

15. Laboratories should run a full revalidation (equivalent to initial analytic validation) when the antibody clone is changed for an existing validated assay."

The American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP)

The American Society of Clinical Oncology and the College of American Pathologists currently recommend that "all newly diagnosed patients with breast cancer must have a HER2 test performed" (Wolff et al., 2013). Also, for those who develop metastatic disease, a HER2 test must be done on tissue from the metastatic site, if available. In less common HER2 breast cancer patterns, as observed in approximately 5% of cases by dual-probe in situ hybridization (ISH) assays, new recommendations have been made to make a final determination of positive or negative HER2 tissue. This new "diagnostic approach includes more rigorous interpretation criteria for ISH and requires concomitant IHC review for dual-probe ISH groups… to arrive at the most accurate HER2 status designation (positive or negative) based on combined interpretation of the ISH and IHC assays;" further, "The Expert Panel recommends that laboratories using single-probe ISH assays include concomitant IHC review as part of the interpretation of all single-probe ISH assay results" (Wolff et al., 2018).

The 2018 update included the following changes from the prior 2013 update, particularly focusing on infrequent HER2 test results that were of "uncertain biologic or clinical significance":

- "Revision of the definition of IHC 2+ (equivocal) to the original FDA-approved criteria.
- Repeat HER2 testing on a surgical specimen if the initially tested core biopsy is negative is no longer stated as mandatory. A new HER2 test ***may*** (no longer ***should***) be ordered on the excision specimen on the basis of some criteria (such as tumor grade 3).
- A more rigorous interpretation criteria of the less common patterns that can be seen in about 5% of all cases when HER2 status in breast cancer is evaluated using a dual-probe ISH testing. These cases, described as ISH groups 2 to 4, should now be assessed using a diagnostic approach that includes a concomitant review of the IHC test, which will help the pathologist make a final determination of the tumor specimen as HER2 positive or negative.

The Expert Panel also preferentially recommends the use of dual-probe instead of single-probe ISH assays, but it recognizes that several single-probe ISH assays have regulatory approval in many parts of the world" (Wolff et al., 2018). The 2018 recommendations were affirmed in 2023 (Wolff et al., 2023).

The National Cancer Coalition Network

The NCCN has made numerous recommendations for use of IHC to diagnose and manage various types of cancer. Cancers with clinically useful IHC applications include breast, cervical, various leukemias, and colorectal cancer.

The NCCN states that the determination of estrogen receptor, progesterone receptor, and HER2 status for breast cancer is recommended and may be determined by IHC (NCCN, 2024). Specifically, the guidelines state that "the NCCN Panel endorses the CAP protocol for pathology reporting and endorses the ASCO CAP recommendations for quality control performance of HER2 testing and interpretation of IHC and ISH results." They also specifically endorse the ASCO/CAP HER2 testing guideline "Principles of HER2 testing," and state "HR testing (ER and PR) by IHC should be performed on any new primary or newly metastatic breast cancer using methodology outlined in the latest ASCO/CAP HR testing guideline." Additionally, "PR testing by IHC on invasive cancers can aid in the prognostic classification of cancers and serve as a control for possible false negative ER results. Patients with ER-negative, PR-

positive cancers may be considered for endocrine therapies, but the data on this group are noted to be limited" (NCCN, 2024).

Further, the NCCN recommendations concerning genetic testing for colorectal cancer state, "The panel recommends that for patients or families where colorectal or endometrial tumor is available, one of three options should be considered for workup: 1) tumor testing with IHC or MSI; 2) comprehensive NGS panel (that includes, at minimum, the four MMR genes and *EPCAM, BRAF,* MSI, and other known familial cancer genes); or 3) germline multi-gene testing that includes the four MMR genes and *EPCAM*. The panel recommends tumor testing with IHC and/or MSI be used as the primary approach for pathology-lab-based universal screening" (NCCN, 2023). More recently, the NCCN has made additional recommendations to individuals diagnosed with any type of hereditary colorectal cancer (CRC) syndrome; these recommendations state that "all individuals newly diagnosed with CRC have either MSI or immunohistochemistry (IHC) testing for absence of 1 of the 4 DNA MMR proteins" (NCCN, 2023).

The European Society of Medical Oncology (ESMO)

The ESMO recommends that for cancers of an unknown primary site, "histology and IHC on good quality tissue specimens are required [III, A]" (Krämer et al., 2023). Particularly in the context for gastrointestinal carcinomas, ESMO states "Immunohistochemical loss of *BRCA1*-associated protein 1 (BAP1) or AT-rich interactive domain-containing protein 1A (ARID1A) can support the diagnosis but the final decision can only be made in conjunction with clinical and radiological findings." Other mentions of IHC in their updated 2023 guidelines did not result in any other updated recommendations (Krämer et al., 2023).

Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

Recently, four clinical IHC biomarker assays (PTEN, RB, MLH1, and MSH2) have been validated for use as biomarkers in a nationwide clinical trial; these assays were then approved by the FDA as laboratory-developed tests to assist in the treatment selection of patients in clinical trials (Khoury et al., 2018). This shows that IHC assays are currently being utilized with molecular tests to assist in therapeutic decisions.

Applicable CPT/HCPCS Procedure Codes

CPT	Code Description

88341	Immunohistochemistry or immunocytochemistry, per specimen; each additional single antibody stain procedure
88342	Immunohistochemistry or immunocytochemistry, per spec; initial single antibody stain
88344	Immunohistochemistry or immunocytochemistry, per specimen; each multiplex antibody stain procedure

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

Evidence-based Scientific References

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Revision History

Revision Date	Summary of Changes
12/04/2024	Reviewed and Updated: Updated the background, guidelines and recommendations, and evidence-based scientific references. Literature review did not necessitate any modifications to coverage criteria

Immunopharmacologic Monitoring of Therapeutic Serum Antibodies

Policy Number: AHS - G2105 - Immunopharmacologic Monitoring of Therapeutic Serum Antibodies	Prior Policy Name and Number, as applicable: • AHS - G2105 - Immunopharmacologic Monitoring of Infliximab, Adalimumab and Other Therapeutic Serum Antibodies • AHS - G2105 - Measurement of Serum Antibodies to Infliximab and Adalimumab • AHS - G2105 - Immunopharmacologic Monitoring of Infliximab and Adalimumab
Initial Presentation Date: 09/18/2015 Effective Date: February 1, 2025	

Policy Description

To manage loss of response due to the development of anti-drug antibodies, immunopharmacologic monitoring of circulating drug and anti-drug antibody levels has been proposed. The presence of anti-drug antibodies may promote adverse effects and diminish drug efficacy (Bendtzen, 2024; Tighe & McNamara, 2017).

Targeted inhibitors of tumor necrosis factor-alpha (TNF) are widely used in the treatment of several inflammatory conditions, including rheumatoid arthritis (RA), spondyloarthritis, inflammatory bowel disease, and psoriasis. Some of these targeted inhibitors include, but are not limited to, infliximab, adalimumab, etanercept, and golimumab (Bendtzen, 2024).

Related Policies

Policy Number	Policy Title
AHS-G2098	Immune Cell Function Assay
AHS-G2155	General Inflammation Testing

Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in "Applicable State and Federal Regulations" section of this policy document.

1) For individuals with inflammatory bowel disease (IBD), drug and/or antibody concentration testing once every two weeks for anti-tumor necrosis factor (anti-TNF) therapies, vedolizumab therapy, or ustekinumab therapy **MEETS COVERAGE CRITERIA.**

   a)

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

2) For individuals with conditions other than IBD (e.g., spondyloarthritis, rheumatoid arthritis, psoriatic arthritis, and psoriasis), drug and/or antibody concentration testing for anti-TNF therapies **DOES NOT MEET COVERAGE CRITERIA**.

3) For all other situations not addressed above, measurement of the serum drug levels **and/or** measurement of the antibodies to the drugs **DOES NOT MEET COVERAGE CRITERIA** for any of the following drugs (alone or as a combination test):

   a) adalimumab

   b) certolizumab

   c) etanercept

   d) golimumab

   e) infliximab

   f) infliximab-dyyb

   g) infliximab-abda

   h) rituximab

   i) ustekinumab

   j) vedolizumab

Table of Terminology

Term	Definition
AAA	Antibodies against adalimumab
AACC	American Association for Clinical Chemistry

ACG	American College of Gastroenterology
ADA	Adalimumab
ADAbs	Anti-drug antibody status
AGA	American Gastroenterological Association
anti-TNF	Anti-tumor necrosis factor
ATA	Antibodies-to-adalimumab
ATI	Antibodies-to-infliximab
ATI-HMSA	Homogeneous mobility shift assay
bDMARDs	Biologic disease-modifying antirheumatic drugs
CD	Crohn's Disease
CER	Certolizumab
CLIA '88	Clinical Laboratory Improvement Amendments of 1988
CMS	Centers for Medicare and Medicaid
DBS	Dried blood spots
ELISA	Enzyme- linked immunosorbent assay
FDA	Food and Drug Administration
GOL	Golimumab
HMSA	Homogeneous mobility shift assay
IBD	Inflammatory bowel disease
IFX	Infliximab
LabCorp	Laboratory Corporation of America Holdings
LDTs	Laboratory developed tests
LFA	Lateral flow-based assay
NICE	National Institute for Health and Clinical Excellence
non-TDM	Non-therapeutic drug monitoring
OH	Ohio
pTDM	Proactive therapeutic drug monitoring
QI	Quality improvement
RA	Rheumatoid arthritis
RR	Risk ratio
TC	Trough concentration
TDM	Therapeutic drug monitoring
TNF	Tumor necrosis factor
UC	Ulcerative colitis
UST	Ustekinumab
VED	Vedolizumab

Scientific Background

Tumor necrosis factor (TNF) inhibitors competitively inhibit the binding of TNF to its receptors, reducing inflammation and halting disease progression (Lis et al., 2014). They are used for treatment of inflammatory conditions, including rheumatoid arthritis (RA), psoriatic arthritis, juvenile arthritis, inflammatory bowel disease (Crohn's and ulcerative colitis), and ankylosing spondylitis (Bendtzen, 2024; Lis et al., 2014). Five primary biologic TNF inhibitors are used for inflammatory diseases; infliximab,

adalimumab, certolizumab pegol, golimumab, and etanercept. However, these inhibitors may lead to the formation of auto-drug antibodies, potentially hindering treatment and causing other adverse effects such as allergic reactions (Bendtzen, 2024).

Tumor necrosis factor inhibitors are a subset of biologic disease-modifying antirheumatic drugs (bDMARDs), which "improve symptoms and reduce structural damage of joints, the gastrointestinal tract, and other affected organs." However, patients oftentimes do not respond to treatment, with upwards of 50% of patients attaining "secondary failure," or inadequate disease control. Important contributors to the secondary failure include anti-drug antibodies and low drug concentrations, which may then contribute to antidrug antibody formation. Generally, the approach to prescribing bDMARDs, such as infliximab, is to adjust or switch "only when there is clinical evidence that remission or low disease activity is not achieved or maintained, which may occur months after treatment initiation." Sometimes, drugs like methotrexate may be prescribed along with the bDMARDs to prevent antidrug antibody development. Guidelines recommending therapeutic drug monitoring (TDM) also vary by inflammatory disease – for example, it is recommended for inflammatory bowel disease (IBD) but not rheumatoid arthritis (RA). To prevent the drawbacks of using bDMARDs from accumulating further, proactive TDM is best supported, but it does not come without barriers like additional personnel needed for constant monitoring, and a dearth of understanding of how other bDMARDs are affected similarly or differently (Wallace & Sparks, 2021).

Most TNF inhibitors are given to individuals in a step wise manner, utilizing an induction period, whereby medication is given more frequently at the beginning of treatment, with frequency of drug delivery often decreasing following the initial induction period. The standard induction period for infliximab is intravenous drug delivery at zero, two, and six weeks, with maintenance therapy occurring every eight weeks. In contrast, adalimumab is given subcutaneously at week zero, week two, and week four, then every other week thereafter as maintenance therapy. Certolizumab induction is subcutaneous delivery at week zero, week two, and week four, then every four weeks for maintenance therapy. Individuals receiving treatment should receive therapeutic drug monitoring to ensure proper response to the dose of the medication and to the medication itself. The drug trough level (the lowest level of the drug in the individuals system) should be assessed no more than 24 hours prior to the next scheduled dose of the drug (Lichtenstein, 2024).

Additional biologics are approved for the treatment of IBD (ustekinumab and vedolizumab) and are often recommended as alternatives to TNF inhibitors. However, similar to the therapeutic drug monitoring required for TNF inhibitors, therapeutic drug monitoring is also essential in individuals receiving these biologics. Ustekinumab is given as a one-time intravenous infusion dose for individuals with moderate to severe Crohn disease (CD) or ulcerative colitis (UC); for individuals who respond to the initial dose, maintenance therapy by subcutaneous delivery should occur every eight weeks (Lexidrug™, 2024a). For individuals with CD or UC, vedolizumab is given by intravenous delivery at week zero, week two, and week six, then every eight weeks thereafter when maintenance is performed through intravenous delivery. After the first two intravenous infusions, subcutaneous delivery every two weeks is a viable option during the maintenance period (Lexidrug™, 2024b).

Proprietary Testing

To optimize dosing of TNF inhibitors, therapeutic drug monitoring (TDM) of both these drugs as well as anti-drug antibodies has been proposed. This dual monitoring is thought to help clinicians manage drug regimens for these patients, such as adjusting the dose or changing the drug entirely. Identifying the presence and concentration of these drugs and auto-drug antibodies may help avoid nonresponse to

treatment. Most assays for the assessment of serum antibodies will also report the drug concentration (Lichtenstein, 2024). For example, HalioDx Inc. offers OptimAbs, which a set of assays for eight biologic agents (adalimumab, certolizumab pegol, golimumab, infliximab, infliximab-dyyb, infliximab-abda, ustekinumab, and vedolizumab). These assays are intended to allow providers to monitor, manage response, and optimize dose (Theradiag, 2018). Prometheus Anser also offers a series of assays for assessment of these anti-drug antibodies, with assessments for four biologics (adalimumab, infliximab, ustekinumab, and vedolizumab). They also measure the levels of antibodies against the drug in question (Prometheus Laboratories, 2024). LabCorp offers eight assays for 10 biologics (adalimumab, certolizumab, etanercept, golimumab, infliximab, infliximab- dyyb, infliximab-abda, rituximab, ustekinumab, and vedolizumab) encompassed in one portfolio called "DoseASSURE" (LabCorp, 2024).

Clinical Utility and Validity

Wang et al. (2012) developed and validated a non-radiolabeled homogeneous mobility shift assay (HMSA) to measure the levels of both infliximab and the antibodies-to-infliximab (ATI) ratio in serum samples. The assay was validated for both items and the sample was compared to the traditional enzyme-linked immunosorbent assay (ELISA). Intra- and interassay precision rates for the ATI-HMSA were less than 4% and less than 15%, respectively, and less than 6% and less than 15%, respectively, for the infliximab-HMSA. The lower limit of quantitation of the ATI-HMSA was found to be 0.012 µg/mL in serum and the HMSA correlated well with the ELISA for ATI levels.

Wang et al. (2013) developed and validated a non-radiolabeled HMSA to measure antibodies-to-adalimumab (ATA) and adalimumab levels in serum samples. Analytic validation of performance characteristics (calibration standards, assay limits, et al.) was performed for both the ATA- and adalimumab-HMSA. Because the elimination half-life of adalimumab (10-20 days) overlaps the dosing interval (every two weeks) and thus the drug-free interval for antibody formation is small, ATA-positive sera samples for calibration standards were difficult to collect from human patients. Instead, antisera from rabbits immunized with adalimumab were pooled to form calibration standards. Serial dilutions of these ATA calibration standards then generated a standard curve against which test samples were compared. With over 29 experimental runs, intra-assay precision and accuracy for the adalimumab-HMSA was <20% and <3%, respectively; interassay (run-to-run, analyst-to-analyst, and instrument-to-instrument) precision and accuracy were less than 12% and less than 22%, respectively. For the ATA-HMSA, variance for intra-assay precision and accuracy were less than 3% and less than 13%, respectively; variance for interassay precision and accuracy were less than 9% and less than 18%, respectively (Wang et al., 2013). ELISA could not be used as a standard comparator due to competition from circulating drug.

Van Stappen et al. (2016) validated a rapid, lateral flow-based assay (LFA) for quantitative determination of infliximab and to assess thresholds associated with mucosal healing in patients with ulcerative colitis. They found that the LFA agreed well with the traditional ELISA for quantification of infliximab with correlation coefficients of 0.95 during induction. A trough concentration (TC) of ≥2.1 µg/ml was associated with mucosal healing. They concluded that "with a time-to-result of 20 min, individual sample analysis and user-friendliness, the LFA outplays ELISA as a rapid, accurate tool to monitor infliximab concentrations" (Van Stappen et al., 2016).

Steenholdt et al. (2014) investigated "the cost-effectiveness of interventions defined by an algorithm designed to identify specific reasons for therapeutic failure." A total of 69 patients with secondary infliximab (IFX) failure were randomized either to IFX dose intensification (n = 36) or interventions based on serum IFX and IFX antibody levels (n = 33). The researchers found that "Costs for intention-to-treat

patients were substantially lower (34%) for those treated in accordance with the algorithm than by infliximab (IFX) dose intensification: €6038 vs €9178. However, disease control, as judged by response rates, was similar: 58% and 53%, respectively" (Steenholdt et al., 2014). They concluded that "treatment of secondary IFX failure using an algorithm based on combined IFX and IFX antibody measurements significantly reduces average treatment costs per patient compared with routine IFX dose escalation and without any apparent negative effect on clinical efficacy" (Steenholdt et al., 2014).

Roblin et al. (2014) conducted a prospective study of 82 patients with inflammatory bowel disease (IBD) having a disease flare while being on adalimumab (ADA) 40 mg every two weeks. All patients were primary responders to ADA therapy and were anti-TNF I. ADA trough levels and antibodies against ADA (AAA) were measured. All patients were optimized with ADA 40 mg weekly. Four months later, in the absence of clinical remission, patients were treated with infliximab. The researchers concluded, "The presence of low ADA trough levels without AAA is strongly predictive of clinical response in 67% of cases after ADA optimization. Conversely, low ADA levels with detectable AAA are associated with ADA failure, and switching to IFX should be considered. ADA trough levels >4.9 µg/ml are associated with failure of two anti-TNF agents (ADA and IFX) in 90% of cases and switching to another drug class should be considered" (Roblin et al., 2014).

Mitchell et al. (2016) studied if IFX TDM allows for objective decision making in patients with IBD and loss of response. A total of 71 patients with IBD that had IFX TDM were examined, and their serum concentration of anti-drug antibodies were measured. Patients were grouped by TDM results and changes in management were examined due to groupings: group one, low IFX/high ADA; group two, low IFX/low ADA; group three, therapeutic IFX. Of the 71 patients, 37% underwent an "appropriate" change in therapy based on group. The authors concluded that "a trend towards increased remission rates was associated with appropriate changes in management following TDM results. Many patients with therapeutic IFX concentrations did not undergo an appropriate change in management, potentially reflecting a lack of available out-of-class options at the time of TDM or due to uncertainty of the meaning of the reported therapeutic range" (Mitchell et al., 2016).

Barlow et al. (2016) evaluated the clinical utility of antibodies in relation to C-reactive protein concentrations. A total of 108 patients contributed 201 samples, and total anti-infliximab antibodies were measured in 164 samples. The authors found that median trough infliximab was 3.7 µg / mL, and 23% of the samples were ≤1 µg / mL. They also noted that "Serum C-reactive protein was found to be significantly higher where infliximab was ≤1 compared to >1 µg/mL," but no "strict" correlation was seen (Barlow et al., 2016). Approximately 85% of samples with positive anti-infliximab antibodies had infliximab ≤1 µg / mL and the authors concluded that "our findings support measurement of anti-infliximab antibodies only in the context of low infliximab concentrations <1 µg/mL. A higher therapeutic cut-off may be relevant in patients with negative antibodies. Further work is indicated to investigate the clinical significance of positive antibodies with therapeutic infliximab concentrations" (Barlow et al., 2016).

Moore et al. (2016) performed a systematic review and meta-analysis of studies that reported serum infliximab levels according to IBD outcomes. Twenty-two studies were examined, encompassing 3483 patients. Twelve studies reported IFX levels in a manner "suitable" for estimating the effect. The researchers found that "During maintenance therapy, patients in clinical remission had significantly higher mean trough IFX levels than patients not in remission: 3.1 µg/ml versus 0.9 µg/ml. The standardized mean difference in serum IFX levels between groups was 0.6 µg/ml. Patients with an IFX level > 2 µg/ml were more likely to be in clinical remission (risk ratio [RR]: 2.9), or achieve endoscopic remission [RR 3] than patients with levels < 2 µg/ml." The study concluded, "There is a significant

difference between serum infliximab levels in patients with IBD in remission, compared with those who relapse. A trough threshold during maintenance > 2 µg/ml is associated with a greater probability of clinical remission and mucosal healing" (Moore et al., 2016).

Wang et al. (2018) submitted an abstract to the 2018 Therapeutic Drug Management and Toxicology Division Abstract Competition on July 30, 2018, conducted by the American Association for Clinical Chemistry (AACC). This abstract focused on InformTx's assays for TDM and the authors reviewed TDM results for six biologics: adalimumab (ADA), certolizumab (CER), golimumab (Syversen et al.), infliximab (IFX), ustekinumab, and vedolizumab (VED). A total of 18837 sera samples were analyzed with InformTx's assays and patients' responses were predicted based on drug and anti-drug antibody status (ADAbs). The need for drug optimization was assessed by comparing patient drug levels to recommended therapeutic drug levels and laboratory defined higher ADAbs. The authors found that "64.1%, 30.2%, 83.9%, 60.4%, 25.2%, and 69.1% of the patients treated with ADA, CER, GOL, INF, UST, and VED, respectively, had drug level equal to or greater than the recommended therapeutic level and undetectable ADAbs." Approximately 4.5%-33% patients had a drug concentration above the recommended therapeutic level. In contrast, patients (31.0% in ADA, 57.0% in CER, 12.1% in GOL, 32.5% in INF, 74.4% in UST, and 30.6% in VED) had undetectable or suboptimal levels of drugs and undetectable or lower levels of ADAbs (Wang et al., 2018).

Fernandes et al. (2019) examined whether TDM can improve clinical outcomes in Crohn's disease (CD) and ulcerative colitis (UC) patients. A total of 205 patients were included in the study, and 56 patients were placed in a "proactive" regimen. This proactive regimen involved measuring infliximab (IFX) trough levels and antidrug antibodies before the fourth infusion and subsequently every two infusions. The regimen aimed to establish an IFX trough level of 3-7 ug/mL for CD patients and 5-10 ug/mL for UC patients. The control group was made of patients treated with IFX but without TDM. The authors found that treatment escalation was more common in the proactive TDM (pTDM) group (76.8% vs 25.5%), mucosal healing was more common (73.2% vs 38.9%), and surgery was less common (8.9% vs 20.8%). Proactive TDM also decreased the odds of any unfavorable outcome by an odds ratio of 0.358. The authors concluded that "Proactive TDM is associated with fewer surgeries and higher rates of mucosal healing than conventional non-TDM-based management" (Fernandes et al., 2019).

Negoescu et al. (2019) performed a cost-effectiveness analysis of proactive verses reactive TDM in a simulated population of individuals with CD on IFX. The proactive strategy measured IFX concentration and antibody status every six months, or at the time of a flare, then dosed IFX appropriately. The reactive strategy measured both IFX concentration and antibodies at the time of a flare. The authors found that the proactive strategy led to fewer flares, finding an "incremental cost-effectiveness ratio of $146,494 per quality-adjusted life year." More patients stayed on IFX in the proactive strategy (63.4% vs 58.8% at year five). The authors concluded that "assuming 40% of the average wholesale acquisition cost of biologic therapies, proactive TDM for IFX is marginally cost-effective compared with a reactive TDM strategy. As the cost of infliximab decreases, a proactive monitoring strategy is more cost-effective" (Negoescu et al., 2019).

Papamichael, Juncadella, et al. (2019) studied the therapeutic drug monitoring of adalimumab in populations with IBD. This multicenter retrospective cohort study included data from 382 patients with IBD (including 311 patients with CD). Participants received either standard of care or at least one proactive TDM. "Multiple Cox regression analyses showed that at least one proactive TDM was independently associated with a reduced risk for treatment failure" (Papamichael, Juncadella, et al., 2019). This study shows that proactive TDM of adalimumab may help to decrease rates of treatment failure for IBD patients.

In February 2016, Guido et al. (2020) developed quality improvement (QI) methods to improve post-induction TDM in pediatric IBD patients initiating anti-TNF therapy at the Nationwide Children's Hospital in Columbus, OH. They implemented interventions to improve TDM using the Institute for Healthcare Improvement Plan-Do-Study-Act cycle approach. Their QI approaches improved post-induction anti-TNF TDM from a baseline off 43% in 2015 to greater than 80% by the end of 2017. Specifically, infliximab post-induction TDM and adalimumab post-induction TDM improved from a baseline of 59% to 89% and 14% to 79%, respectively. Most importantly, they note that "subtherapeutic post-induction infliximab levels were common, indicating a strong need for anti-TNF TDM and an opportunity for dose optimization."

Syversen et al. (2021) studied the therapeutic drug monitoring of infliximab in populations with immune-mediated inflammatory disease. Proactive therapeutic drug monitoring (TDM) as an alternative to standard therapies was proposed to treat patients safely and effectively during biologic drug therapies, specifically, in this study, patient populations who were prescribed Infliximab. A randomized, parallel-group and open-label clinical trial was established with a total of 458 adults with the diagnosis of rheumatoid arthritis, spondyloarthritis, psoriatic arthritis, ulcerative colitis, Crohn's disease, or psoriasis. All patients participating in Infliximab maintenance therapy were from a selection of Norwegian hospitals. Routine monitoring of serum drug levels and antidrug antibodies was performed on a randomized 1:1 basis (i.e. some patients received standard therapy, while others received scheduled monitoring of serum drug levels and anti-TNF antibodies). The primary outcome of sustained disease control without disease worsening was evident in 167 patients, which comprised 73.6% of the therapeutic drug monitoring cohort. A total of 127 patients in the standard therapy group (55.9%) showed sustained disease control outcomes. This comprised an "estimated adjusted difference" of 17.6% between the two groups. In conclusion, the authors stated that they found "proactive TDM was more effective than treatment without TDM in sustaining disease control without disease worsening. Further research is needed to compare proactive TDM with reactive TDM, to assess the effects on long-term disease complications, and to evaluate the cost-effectiveness of this approach."

Cox et al. (2021) conducted a retrospective review of rheumatology patients who had antidrug antibody levels tested between October 2015 and April 2019 in order to assess the reasons for and outcomes in patients on adalimumab or infliximab. From the 237 patients included on the analysis, most patients were tested due to "clinical evidence of a flare in disease" and "patient reported worsening of symptoms." A total of 38% changed biologics and 2% had dosing schedules changed, which is consistent with the 30-40% failure rate of response to first-line biologics. It was also found that "those with strongly positive antibodies were more likely to switch biologics than those with normal antibodies (84% vs 28%, p =0.01)," and that "patients with clinically active disease but normal antibodies and drug levels were more likely to switch biologics than patients with no evidence of active disease but positive antibodies (p=0.03)." This demonstrates the benefit of antidrug antibody level monitoring on informing treatment among specific patient populations (Cox et al., 2021).

Pan et al. (2022) utilized drug concentrations of infliximab, adalimumab, and ustekinumab in patients with postoperative Crohn's disease to investigate the impact on clinical outcomes. From 130 patients, the researchers found that in patients treated with infliximab with ≥3μg/mL and in patients treated with adalimumab ≥7.5μg/mL, "higher rates of deep remission existed," and similar differences were found for both clinical and objective remission. However, for ustekinumab, "clinical and objective remission were similar between patients regardless of drug concentration." These conclusions demonstrated that "established anti-tumor necrosis factor concentrations" could inform the rationale behind clinical improvement for certain patients that suffer from diseases that lack prior data to support the positive use of bDMARDs (Pan et al., 2022).

Guidelines and Recommendations

National Institute for Health and Clinical Excellence (NICE)

The 2016 Guidelines for therapeutic monitoring of TNF-alpha inhibitors in Crohn's disease stated that "enzyme-linked immunosorbent assay (ELISA) kits show promise for therapeutic monitoring of TNF-alpha inhibitors in people with Crohn's disease but there is insufficient evidence to recommend their routine adoption" (NICE, 2016).

The NICE also states that use of ELISA tests should be a part of research and/or data collection and that more research is needed to determine the clinical effectiveness of ELISA tests for therapeutic monitoring of TNF-alpha inhibitors for rheumatoid arthritis. "Enzyme-linked immunosorbent assay (ELISA) tests for therapeutic monitoring of tumour necrosis factor (TNF)-alpha inhibitors (drug serum levels and antidrug antibodies) show promise but there is currently insufficient evidence to recommend their routine adoption in rheumatoid arthritis. The ELISA tests covered by this guidance are Promonitor, IDKmonitor, LISA-TRACKER, RIDASCREEN, MabTrack, and tests used by Sanquin Diagnostic Services" (NICE, 2019).

American Gastroenterological Association

The AGA published guidelines on Therapeutic Drug Monitoring in Inflammatory Bowel Disease recommending:

"In adults with active IBD treated with anti-TNF agents, the AGA suggests reactive therapeutic drug monitoring to guide treatment changes. Conditional recommendation, very low quality of evidence" (Feuerstein et al., 2017).

In adult patients with quiescent IBD treated with anti-TNF agents, the AGA makes no recommendation regarding the use of routine proactive therapeutic drug monitoring (Feuerstein et al., 2017).

A technical report released by the AGA in the same year noted that for patients with quiescent IBD being treated with anti-TNF agents, the benefit of routine proactive TDM was "uncertain" compared to no monitoring. However, they observe a potential benefit for reactive TDM (Vande Casteele et al., 2017).

American College of Rheumatology and National Psoriasis Foundation Guideline for the Treatment of Psoriatic Arthritis

These guidelines do not mention monitoring of TNF inhibitors for antidrug antibodies or TNF inhibitor levels (Singh et al., 2019).

American College of Gastroenterology (ACG)

The ACG released an update regarding management of Crohn's Disease (CD), stating that "if active CD is documented, then assessment of biologic drug levels and antidrug antibodies (therapeutic drug monitoring) should be considered" (Lichtenstein et al., 2018).

The ACG published guidelines on management of ulcerative colitis. In it, they observe that "the patient with nonresponse or loss of response to therapy should be assessed with therapeutic drug monitoring to identify the reason for lack of response and whether to optimize the existing therapy or to select an alternate therapy." However, they remark that there is "insufficient evidence" to support a benefit for proactive TDM in "all unselected patients with UC in remission" (Rubin et al., 2019).

Consensus Statement on Therapeutic Drug Monitoring of Biologic Agents for Patients With IBD

A consensus statement on appropriate therapeutic drug monitoring for IBD patients has been published. This statement was published in the journal of Clinical Gastroenterology and Hepatology, which is published by Elsevier on behalf of the AGA. A total of 28 statements were provided to a 13-member panel, and 24 of these statements reached a consensus. All statements were rated on a scale of one to ten, and statements were accepted if 80% or more of the participants agreed with a score ≥ seven. All 28 statements are shown below. Overall, "For anti-tumor necrosis factor (anti-TNF) therapies, proactive TDM was found to be appropriate after induction and at least once during maintenance therapy, but this was not the case for the other biologics. Reactive TDM was appropriate for all agents both for primary non-response and secondary loss of response. The panelists also agreed on several statements regarding TDM and appropriate drug and anti-drug antibody concentration thresholds for biologics in specific clinical scenarios" (Papamichael, Cheifetz, et al., 2019).

"**Table 4:** Scenarios of Applying Therapeutic Drug Monitoring of Biological Therapy in Patients with Inflammatory Bowel Disease

Anti-TNFs

1. It is appropriate to order drug/antibody concentration testing in responders at the end of induction for all anti-TNFs. 92 (12/13)
2. It is appropriate to order drug/antibody concentration testing at least once during maintenance for patients on all anti-TNFs. 100 (13/13)
3. It is appropriate to order drug/antibody concentration testing of anti-TNFs at the end of induction in primary non-responders. 100 (13/13)
4. It is appropriate to order drug/antibody concentration testing for all anti-TNFs in patients with confirmed secondary loss of response. 100 (13/13)

Vedolizumab

5. It is appropriate to order drug/antibody concentration testing for vedolizumab in responders at the end of induction. 15 (2/13)a
6. It is appropriate to order drug/antibody concentration testing at least once during maintenance for patients on vedolizumab. 46 (6/13)a
7. It is appropriate to order drug/antibody concentration testing for vedolizumab in non-responders at the end of induction. 92 (12/13)
8. It is appropriate to order drug/antibody concentration testing for vedolizumab in patients with confirmed secondary loss of response. 83 (10/12)

Ustekinumab

9. It is appropriate to order drug/antibody concentration testing for ustekinumab in responders at the end of induction. 39 (5/13)a
10. It is appropriate to order drug/antibody concentration testing at least once during maintenance for patients on ustekinumab. 31 (4/13)a
11. It is appropriate to order drug/antibody concentration testing for ustekinumab in non-responders at the end of induction (at 8 weeks). 92 (12/13)
12. It is appropriate to order drug/antibody concentration testing for ustekinumab in patients with confirmed secondary loss of response. 83 (10/12)" (Papamichael, Cheifetz, et al., 2019)

Table 5: Biological Drug Concentrations and Anti-Drug Antibodies When Applying Therapeutic Drug Monitoring in Inflammatory Bowel Disease

General

13. There is no difference in indication for ordering drug/antibody concentrations or interpretation of results for biosimilars or the originator drug. 100 (13/13)
14. The threshold drug concentration may vary depending on disease phenotype and desired therapeutic outcome. 100 (13/13)
15. In the presence of adequate trough drug concentrations, anti-drug antibodies are unlikely to be clinically relevant. 100 (12/12)
16. Other than for anti-infliximab antibodies, there are not enough data to recommend a threshold for high anti-drug antibody titers for the biologic drugs. 100 (12/12)

Infliximab

17. The current evidence suggests that the variability of infliximab concentrations between the different assays is unlikely to be clinically significant. 100 (13/13)a
18. There is insufficient evidence that inter-assay drug concentration results are comparable for biologic drugs other than for infliximab. 100 (13/13)
19. The minimal trough concentration for infliximab post-induction at week 14 should be greater than 3 µg/mL, and concentrations greater than 7 µg/mL are associated with an increased likelihood of mucosal healing. 100 (13/13)
20. During maintenance the minimal trough concentration for infliximab for patients in remission should be greater than 3 µg/mL. For patients with active disease, infliximab should generally not be abandoned unless drug concentrations are greater than 10 µg/mL. 92 (12/13)
21. In the absence of detectable infliximab, high titer anti-infliximab antibodies require a change of therapy. Low level antibodies can sometimes be overcome. For the ANSER assay, a high titer anti-infliximab antibody at trough is defined as 10 U/mL, for RIDAscreen the cutoff is 200 ng/mL, and for InformTx/Lisa Tracker the cutoff is 200 ng/mL. For other assays, there are insufficient data to define an adequate cutoff for a high titer anti-infliximab antibody. 100 (13/13)

Adalimumab

22. The minimum drug concentration at week 4 for adalimumab should at least be 5 µg/mL. Drug concentrations greater than 7 µg/ml are associated with an increased likelihood of mucosal healing. 83 (10/12)a
23. During maintenance the minimum trough concentration for adalimumab for patients in remission should be greater than 5 µg/mL. For patients with active disease, adalimumab should generally not be abandoned unless drug concentrations are greater than 10 µg/mL. 100 (12/12)

Certolizumab pegol

24. The minimum concentrations for certolizumab pegol at week 6 should be greater than 32 µg/mL. 100 (12/12)
25. During maintenance the minimum trough concentration for certolizumab pegol for patients in remission should be 15 µg/mL. 92 (11/12)

Golimumab

26. The minimum drug concentration at week 6 for golimumab should at least be 2.5 µg/mL. 92 (11/12)
27. During maintenance the minimum trough concentration for golimumab for patients in remission should be greater than 1 µg/mL. 92 (11/12)

Vedolizumab/ustekinumab

28. Although there are emerging data that may show an association between drug concentrations and outcomes, they are not sufficient to guide specific induction and maintenance drug concentrations for vedolizumab and ustekinumab other than confirming that there is detectable drug. 100 (12/12)" (Papamichael, Cheifetz, et al., 2019)

Consensus Statement Regarding the Clinical Utility of TDM for Biologics in Inflammatory Bowel Disease (IBD).

A comprehensive literature review was performed regarding "TDM of biologic therapies in IBD and 45 statements were subsequently formulated on the potential application of TDM in IBD. The statements, along with literature, were then presented to a panel of 10 gastroenterologists with expertise in IBD and TDM who anonymously rated them on a scale of 1 to 10 (1=strongly disagree and 10=strongly agree)" (Cheifetz et al., 2021).

Table 1.

Statements regarding reactive therapeutic drug monitoring of biologics

Statement	Vote agreement, %	Strength of recommendation
1. Reactive TDM should be performed in patients with confirmed primary non-response to anti-TNF therapy.	100	9.7
2. Reactive TDM should be performed in patients with confirmed secondary loss of response to anti-TNF therapy.	100	9.8
3. Reactive TDM has been proven more cost-effective than empiric anti-TNF therapy optimization.	100	8.6
4. When performing reactive TDM for secondary loss of response to infliximab, treatment discontinuation should not be considered until a drug concentration of at least 10-15µg/ml is achieved.	90	8.5
5. When performing reactive TDM for secondary loss of response to adalimumab, treatment discontinuation should not be considered until a drug concentration of at least 10-15µg/ml is achieved.	90	8.3
6. Reactive TDM should be performed in patients with confirmed primary non-response to vedolizumab prior to switching therapy.	100	8.3
7. Reactive TDM should be performed in patients with confirmed primary non-response to ustekinumab prior to switching therapy.	90	7.4

Statement	Vote agreement, %	Strength of recommendation
8. Reactive TDM should be performed in patients with confirmed secondary loss of response to vedolizumab.	100	8.9
9. Reactive TDM should be performed in patients with confirmed secondary loss of response to ustekinumab.	90	8.5

Table 2.

Statements regarding proactive therapeutic drug monitoring of biologics.

Statement	Vote agreement, %	Strength of recommendation
10. Proactive TDM should be performed post induction for patients treated with anti-TNF therapy.	90	9
11. Proactive TDM should be performed at least once during maintenance therapy for patients treated with anti-TNF therapy.	90	8.8
12. Proactive TDM should be utilized after reactive TDM of anti-TNF therapy.	80	8.1
13. Proactive TDM is most important in more severely active patients and in patients who have higher drug clearance.	90	8.5
14. When infliximab de-escalation (dose reduction) is considered in patients in remission, proactive TDM both prior to and after de-escalation should be performed.	100	9.2
15. Proactive TDM for optimizing anti-TNF monotherapy is better than unoptimized anti-TNF monotherapy.	100	9
16. Proactive TDM for optimizing anti-TNF monotherapy in select patients is an alternative to combination anti-TNF therapy with an immunomodulator.	90	8.5
17. More data are needed to support the use of proactive TDM for biologics other than anti-TNF therapies.	100	9.2

Table 3.

General statements regarding therapeutic drug monitoring of biologics.

Statement	Vote agreement, %	Strength of recommendation
18. There is clinical utility for TDM to be performed in patients treated with anti-TNF therapy during induction.	80	8

Statement	Vote agreement, %	Strength of recommendation
19. Increased anti-TNF clearance is associated with anti-drug antibodies, male gender, low albumin, high baseline CRP and high BMI.	90	9.2
20. TDM (drug concentration and antibodies to infliximab) should be performed following a drug holiday in patients treated with infliximab prior to second dose after re-starting.	100	9
21. Patients should be followed over time with the same TDM assay, if possible, until commercial assays are accurately cross-validated and standardized.	80	8.1
22. There are no differences in performing and interpreting the results of TDM between biosimilars and originator biologic drugs.	100	9.4

Table 4.

Statements regarding immunogenicity of biologics.

Statement	Vote agreement, %	Strength of recommendation
23. Anti-drug antibodies are more clinically relevant when trough drug concentrations are undetectable.	90	9.1
24. Patients with secondary loss of response to anti-TNF therapy due to the development of high-titer anti-drug antibodies should not be dose-escalated, but instead should be switched to a different therapy (within-class or out of class).	100	9.4
25. When considering switching within drug class in case of secondary loss of response to a first anti-TNF drug due to the development of anti-drug antibodies, an immunomodulator should be added to a subsequent anti-TNF therapy.	90	8.5
26. All commercially available assays are appropriate to use for TDM, however, for antibody measurement, beyond the homogeneous mobility shift assay there are not sufficient data to support specific clinically relevant cut-offs to define high-titer antibodies.	100	8.3
27. Low-titer antibodies to infliximab can be defined as <10 U/ml for the homogeneous mobility shift assay.	90	8.1
28. Low titer anti-drug antibodies can be overcome by treatment optimization (dose escalation, dose interval shortening and/or addition of an immunomodulator).	100	8.4
29. The formation of antibodies to infliximab or adalimumab can be reduced by the use of immunomodulators.	100	9.1

Statement	Vote agreement, %	Strength of recommendation
30. HLA-DQA1*05 is associated increased risk of development of antibodies to infliximab and adalimumab.	100	9.3
31. Vedolizumab is associated with less immunogenicity than anti-TNFs.	100	9.2
32. Ustekinumab is associated with less immunogenicity than anti-TNFs.	100	9.9

Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
80145	Adalimumab
80230	Infliximab
80280	Vedolizumab
80299	Quantitation of therapeutic drug, not elsewhere specified
82397	Chemiluminescent assay
84999	Unlisted chemistry procedure
0514U	Gastroenterology (irritable bowel disease [IBD]), immunoassay for quantitative determination of adalimumab (ADL) levels in venous serum in patients undergoing adalimumab therapy, results reported as a numerical value as micrograms per milliliter (µg/mL) Proprietary test: Procise ADL™ Lab/Manufacturer: ProciseDx Inc
0515U	Gastroenterology (irritable bowel disease [IBD]), immunoassay for quantitative determination of infliximab (IFX) levels in venous serum in patients undergoing infliximab therapy, results reported as a numerical value as micrograms per milliliter (µg/mL) Proprietary test: Procise IFXT™

	Lab/Manufacturer: ProciseDx Inc

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

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Revision History

Review Date	Summary of Changes
09/04/2024	Reviewed and Updated: Updated background, guidelines, and evidence-based scientific references. Literature review necessitated the following changes in coverage criteria: CC1 and 2 combined and edited for clarity on frequency of allowed TDM based on guideline recommendations and drug dosing information. CC1 now reads: "1) For individuals with inflammatory bowel disease (IBD), drug and/or antibody concentration testing once every two weeks for anti-tumor necrosis factor (anti-TNF) therapies, vedolizumab therapy, or ustekinumab therapy MEETS COVERAGE CRITERIA." Added CPT code 0514U, 0515U (effective date 10/1/2024)

In Vitro Chemoresistance and Chemosensitivity Assays

Policy Number: AHS – G2100 – In Vitro Chemoresistance and Chemosensitivity Assays	Prior Policy Name and Number, as applicable:
Initial Presentation Date: 09/18/2015 Revision Date: February 1, 2025	

Policy Description

In vitro chemotherapy sensitivity and resistance assays refer to any in vitro laboratory analysis that is performed specifically to evaluate whether tumor growth is inhibited by a known chemotherapy drug or, more commonly, a panel of drugs (Hatok et al., 2009; Schrag et al., 2004).

Related Policies

Policy Number	Policy Title
N/A	

Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

1) In vitro chemosensitivity assays (e.g., histoculture drug response assay, fluorescent cytoprint assay) **DO NOT MEET COVERAGE CRITERIA**.

2) In vitro chemoresistance assays (e.g., extreme drug resistance [EDR] assays) **DO NOT MEET COVERAGE CRITERIA**.

Table of Terminology

Term	Definition
5-FU	5-Fluorouracil
AML	Acute myelocytic leukemia
ASCO	American Society of Clinical Oncology
ATP-CRA	Adenosine triphosphate-based chemotherapy response assay
ATP-TCA	Adenosine triphosphate-tumor chemosensitivity
CDR	Cell death rate
CLIA '88	Clinical Laboratory Improvement Amendments Of 1988
CMS	Centers For Medicare and Medicaid
CR	Complete remission
CSC	Cancer stem cells
DISC	Differential staining cytotoxicity
EDR	Extreme drug resistance
FDA	Food and Drug Administration
HDRA	Histoculture drug response assay
HTCA	Human tumor cell assays
KU	Kinetic units
LCA	Local coverage article
LCD	Local coverage determination
LDT	Laboratory-developed test
MDR	Multiple drug resistance
MiCK	Microculture-kinetic
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolyum Bromide
NCCN	National Comprehensive Cancer Network
OR	Odds ratio
OS	Overall survival
PFS	Progression-free survival
RGCC	Regulator of Cell Cycle
RPPA	Reverse phase protein array
TMZ	Temozolomide

Scientific Background

Chemotherapy treatment recommendation has long been based on carefully designed clinical studies in large patient populations and provide an individual patient with a probability for response based on

clinically observed response rates. This approach has led to major progress in clinical oncology and has helped to identify successful therapeutic regimens for patients with many cancers. However, the response rates are relatively low, and there are still many cancers for which there is only marginal treatment. Tumor cells isolated from these patients often are resistant to a wide range of anticancer drugs. In addition, it is becoming clear that each individual patient's tumor is genotypically and phenotypically different (Hatok et al., 2009).

Chemotherapy sensitivity and resistance assays may also be called human tumor stem cell drug sensitivity assays, tumor stem cell assays, clonogenic or nonclonogenic cytotoxic drug resistance assays, or differential staining cytotoxic assays. These tests were developed to determine if a patient with cancer might be resistant or sensitive to a specific chemotherapy treatment prior to use. A chemosensitivity assay detects the effects (cytotoxic, apoptotic, and so on) of a given chemotherapeutic agent outside an organism. The assays vary, but typically they follow the same steps: cells from the patient are isolated, incubated with the chemotherapeutic agent, and assessed for cell survival and cell response (Hatok et al., 2009; Tatar et al., 2016). This allows clinicians to evaluate the effects of the chemotherapeutic agent without unnecessary exposure to cells. However, there are difficulties with these assays; for example, the potency of a chemotherapeutic agent may only be seen after time has elapsed.

Many assays have been created to assess the potency of chemotherapeutic agents, including proprietary tests such as ChemoFX and ChemoINTEL, as well as non-proprietary assays such as 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolyum bromide (MTT), adenosine triphosphate-tumor chemosensitivity (ATP-TCA), and differential staining cytotoxicity (DISC) (Tatar et al., 2016).

Chemosensitivity assays typically rely on the use of cell cultures within the presence of the anticancer agent(s). For example, the MTT procedure involves culturing tumor cells with anticancer agents, then adding MTT, which is reduced to a blue dye in the cell. The intensity of the uptake allows the user to estimate the drug resistance of the tumor cells. DISC cultures tumor cells in three different concentrations of the drug, incubates them for six days, then uses differential dye staining to identify viable cells (Hatok et al., 2009). Several additional proprietary assays exist, such as ChemoFX (from Helomics), which exposes tumor cells to increasing doses of chemotherapeutic drugs; then, the number of live cells remaining post-treatment is counted. These counts are combined into a dose-response curve, which is used to categorize a tumor's response as "responsive," "intermediate response," or "non-responsive" (Brower et al., 2008). Another proprietary test is the assay from Pierian Biosciences (Grendys et al., 2014; Pierian, 2023). This test relies on drug-induced apoptosis with the quantification of tumor cells' response to chemotherapeutic agents. This test is now branded as ChemoINTEL (Pierian, 2023). A third proprietary test comes from RGCC, marketed as "Onconomics RGCC." This test evaluates both molecular markers and viability assessments to determine efficacy of certain drugs. It follows the same pattern as the previously discussed tests, i.e., developing cell cultures and examining effects of chemotherapeutic agents on their population (RGCC, 2023). Other proprietary assays include human tumor cell assays (HTCA) and human tumor cloning assays.

Another technique is the Extreme Drug Resistance assay (EDR®), which takes cultured cells and exposes them to high concentrations of chemotherapeutic agents for long exposure times. The exposure time to agents for these cells is typically more than 100 times that of what a patient would receive in a regular chemotherapy session. The goal is to isolate the chemotherapeutics that would be of *least* clinical benefit in the treatment process (Karam et al., 2009).

Recent advances have led to new proprietary tests on the market, such as the KIYATEC Inc. ex vivo 3D cell culture technology, which predicts "in vivo cancer drug efficacy through precision ex vivo response

profiling," by using live cancer cells from surgical and/or biopsy specimens to create a tumor specific to the patient genetic profile (KIYATEC, 2023). This manufactured tumor is then used to investigate the patient's potential responses to chemotherapy regimens or drugs. A second new proprietary test, from Theralink, uses a reverse phase protein array (RPPA) test to evaluate over 600 different protein and phosphoprotein targets on a cell's surface. The test is used to evaluate whether FDA-approved cancer therapies and investigational treatments will be effective based on cell surface proteins. Theralink's technology seeks to reduce exposure of patients to cytotoxic treatments and therapies through analysis of drug-protein interactions that drive treatment responses (Theralink, 2023).

Clinical Utility and Validity

Tatar et al. (2016) conducted a study to assess three in vitro chemosensitivity assays in ovarian carcinoma. 26 patients with ovarian carcinoma contributed tumoral tissue, and three assays (the MTT assay, the ATP-TCA assay, and the DISC assay) were used to evaluate the chemosensitivity of paclitaxel, carboplatin, docetaxel, topotecan, gemcitabine, and doxorubicin. The authors stated that all three assays correlated reasonably well with each other and are "particularly useful for serous and advanced cancers." However, they caution that "large prospective studies comparing standard versus assay-directed therapy with an endpoint of overall survival are required before routine clinical utilization of these assays" (Tatar et al., 2016).

Kwon et al. (2016) evaluated the usefulness of the in vitro adenosine triphosphate-based chemotherapy response assay (ATP-CRA) for prediction of clinical response to fluorouracil-based adjuvant chemotherapy in stage II colorectal cancer. Tumor specimens of 86 patients with stage II colorectal adenocarcinoma were tested for chemosensitivity to fluorouracil, and chemosensitivity was determined by cell death rate (CDR) of the drug-exposed cells. In total, 11 of the 86 patients had a recurrence, and the group with CDR ≥20% was associated with better disease-free survival than the group under 20%. The authors concluded that "in stage II colorectal cancer, the in vitro ATP-CRA may be useful in identifying patients likely to benefit from fluorouracil-based adjuvant chemotherapy" (Kwon et al., 2016).

Krivak et al. (2014) conducted an observational study to evaluate if the ChemoFx assay can identify patients who are platinum-resistant prior to treatment. The study included 276 individuals with International Federation of Gynecology and Obstetrics stage III-IV ovarian, fallopian, and peritoneal cancer, and the responsiveness of their tumors was evaluated. All patients were treated with a platinum/taxane regimen following cytoreductive surgery. The authors found that the patients whose tumors were resistant to carboplatin were at increased risk of disease progression compared to those who were nonresistant. The authors stated that "assay resistance to carboplatin is strongly associated with shortened PFS among advanced-stage epithelial ovarian cancer patients treated with carboplatin + paclitaxel therapy, supporting use of this assay [ChemoFx] to identify patients likely to experience early recurrence on standard platinum-based therapy" (Krivak et al., 2014).

Rutherford et al. (2013) conducted a prospective study evaluating the use of ChemoFx assay in recurrent ovarian cancer patients. The study included 252 individuals with persistent or recurrent ovarian cancer and fresh tissue samples were collected for chemoresponse testing. Patients were treated with one of 15 protocol-designated treatments empirically selected by the oncologist, blinded to the assay results. Patients were prospectively monitored for progression-free survival (PFS) and overall survival (OS). Patients treated with an assay-sensitive regimen demonstrated significantly improved PFS and OS while there was no difference in clinical outcomes between intermediate and resistant groups. The researchers concluded that the "study demonstrated improved PFS and OS for patients with either

platinum-sensitive or platinum-resistant recurrent ovarian cancer treated with assay-sensitive agents" (Rutherford et al., 2013).

Hoffman (2018) conducted a study investigating the clinical correlation of histoculture drug response assay (HDRA) in 29 advanced gastric and colon cancer patients. The authors revealed that all 29 were being treated with drugs considered "ineffective" by the HDRA. However, nine patients were also being treated with drugs identified as "effective" by the HDRA, and these patients showed response or arrest of disease progression. The authors investigated another subset of 32 patients treated with mitomycin C and 5-fluorouracil (5-FU) and whom had advanced gastric cancer. Ten patients were identified as "sensitive" to these drugs, and their survival rates were higher than the other 22 whose tumors were "insensitive." A separate 128-patient subset had their tumors evaluated by the HDRA, and the overall and disease-free survival rate was higher for the sensitive group compared to the resistant group. Overall, both "sensitive" groups experienced higher survival rates (Hoffman, 2018).

Strickland et al. (2013) evaluated the correlation of the MiCK assay with patient outcomes in initial treatment of adult acute myelocytic leukemia (AML). 109 patients with untreated AML contributed samples for the MiCK assay. The amount of apoptosis was measured over 48 hours and standardized to "kinetic units" of apoptosis (KU). The authors observed that complete remission (CR) was "significantly" higher in patients with high idarubicin-induced apoptosis (>3 KU) compared to patients with <3 KU. A multivariate analysis indicated the only significant variable to be idarubicin-induced apoptosis. The authors concluded, "Chemotherapy-induced apoptosis measured by the MiCK assay demonstrated significant correlation with outcomes and appears predictive of complete remission and overall survival for patients receiving standard induction chemotherapy" (Strickland et al., 2013).

Howard et al. (2017) developed and assessed a "chemopredictive" assay (ChemoID), which was intended to identify the most effective chemotherapy out of a panel of selected treatments. ChemoID evaluates the efficacy of chemotherapies using a patient's live tumor cells, as well as the cancer stem cells (CSC) that are purported to cause recurrence in patients. The study included 42 glioblastoma patients who were treated with standard of card temozolomide (TMZ). Clinical outcomes such as "tumor response, time to recurrence, progression-free survival (PFS), and overall survival (OS). Odds ratio (OR) associations of 12-month recurrence, PFS, and OS outcomes" were estimated. The authors found that for every 5% increase in CSC kill by TMZ, 12-month patient response (defined as "nonrecurrence of cancer") increased by 2.2-fold. The authors also identified a less significant association with the bulk tumor cells; a 5% increase in bulk tumor cell kill corresponded with a 2.75-fold increase in nonresponse ($p = .07$). At >40% cell kill for CSC and >55% cell kill for bulk tumor cells, the area under curve was 0.989. Median recurrence time was 20 months for patients with a positive (defined as >40%) CSC test, compared to three months for patients with a negative test. Similarly, median recurrence time was 13 months for patients with a positive bulk tumor cell test (>55%), compared to four months for a negative test. Finally, the ChemoID CSC results were found to "potentially" identify more optimal treatments in 34 patients, while the bulk tumor results may have resulted in more optimal treatments in 27 patients. Overall, the authors concluded that "the ChemoID CSC drug response assay has the potential to increase the accuracy of bulk tumor assays to help guide individualized chemotherapy choices" (Howard et al., 2017).

Chen et al. (2018) evaluated in vitro chemosensitivity and multiple drug resistance (MDR) using an ATP-based tumor chemosensitivity assay (ATP-TCA). The authors evaluated 120 lung cancer patients' chemosensitivity to eight single drug chemotherapies and 291 lung cancer patients' chemosensitivity to seven chemotherapy regimens. Additionally, 284 lung adenocarcinoma patients and 90 lung squamous cell carcinoma patients were evaluated for chemosensitivity to both single-drug and chemotherapy regimens. Authors found that "PTX (51.7%), TXT (43.3%), GEM (12.5%), PTX+DDP (62.5%), TXT+L-OHP

(54.3%) and VP-16+DDP (16.2%) had the highest in vitro chemosensitivity rates." Additionally, approximately 37.1% of patients developed resistance to eight single-drug chemotherapies; 25.8% showed resistance to all seven chemotherapy regimens. In conclusion, testing for drug sensitivity before chemotherapy could assist in preventing the "occurrence of primary drug resistance and inappropriate drug treatment" (Chen et al., 2018).

Shuford et al. (2021) investigated whether a direct, live tumor 3D cell-based assay could predict clinical therapeutic response before treatment for patients with high grade glioma. The authors used a 3D cell culture test that was validated for drug concentration, timing, and reproducibility. The 3D cell-based assay predicted the response of patients to temozolomide in 17/20 (85%, P= .007) patients seven days before surgery and before treatment began. Patients who responded to the test had a median over-all survival rate of 11.6 months post-surgery compared with a 5.9-month survival rate (*P*= .0376) for those that did not respond to the cell-based assay. The ex vivo assay also effectively provided evidence for when to use dabrafenib when NGS results did not. The authors noted that the study "both validates the developed assay analytically and clinically and provides case studies of its implementation in clinical practice" (Shuford et al., 2021).

Guidelines and Recommendations

American Society of Clinical Oncology (ASCO)

The 2011 clinical practice guideline update states that: "The use of chemotherapy sensitivity and resistance assays to select chemotherapeutic agents for individual patients is not recommended outside of the clinical trial setting. Oncologists should make chemotherapy treatment recommendations on the basis of published reports of clinical trials and a patient's health status and treatment preferences. Because the in-vitro analytic strategy has potential importance, participation in clinical trials evaluating these technologies remains a priority" (Burstein et al., 2011).

National Comprehensive Cancer Network

The NCCN Practice Guidelines in Oncology for Ovarian Cancer (NCCN, 2023b) state that: "chemosensitivity/resistance and/or other biomarker assays are being used at some NCCN Member Institutions for decisions related to future chemotherapy in situations where there are multiple equivalent chemotherapy options available. The current level of evidence is not sufficient to supplant standard of care chemotherapy." This is a category 3 recommendation (based on any level of evidence but reflects major disagreement).

Chemosensitivity/resistance testing is not mentioned in the guidelines for gastric, colon, or prostate cancers (NCCN, 2023a).

Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
81535	Oncology (gynecologic), live tumor cell culture and chemotherapeutic response by DAPI stain and morphology, predictive algorithm reported as a drug response score; first single drug or drug combination Proprietary test: ChemoFX® Lab/manufacturer: Helomics, Corp
81536	Oncology (gynecologic), live tumor cell culture and chemotherapeutic response by DAPI stain and morphology, predictive algorithm reported as a drug response score; each additional single drug or drug combination (List separately in addition to code for primary procedure) Proprietary test: ChemoFX® Lab/manufacturer: Helomics, Corp
86849	Unlisted immunology procedure
88104	Cytopathology, fluids, washings or brushings, except cervical or vaginal; smears with interpretation
88199	Unlisted cytopathology procedure
88305	Level IV - Surgical pathology, gross and microscopic examination
88313	Special stain including interpretation and report; Group II, all other (eg, iron, trichrome), except stain for microorganisms, stains for enzyme constituents, or immunocytochemistry and immunohistochemistry
88358	Morphometric analysis; tumor (eg, DNA ploidy)
89050	Cell count, miscellaneous body fluids (eg, cerebrospinal fluid, joint fluid), except blood
89240	Unlisted miscellaneous pathology test
0083U	Oncology, response to chemotherapy drugs using motility contrast tomography, fresh or frozen tissue, reported as likelihood of sensitivity or resistance to drugs or drug combinations Proprietary test: Onco4D™ Lab/manufacturer: Animated Dynamics, Inc.
0248U	Oncology (brain), spheroid cell culture in a 3D microenvironment, 12 drug panel, tumor-response prediction for each drug Proprietary test: 3D Predict Glioma Lab/Manufacturer: KIYATEC®, Inc
0249U	Oncology (breast), semiquantitative analysis of 32 phosphoproteins and protein analytes, includes laser capture microdissection, with algorithmic analysis and interpretative report Proprietary test: Theralink® Reverse Phase Protein Array (RPPA) Lab/Manufacturer: Theralink® Technologies, Inc
0285U	Oncology, response to radiation, cell-free DNA, quantitative branched chain DNA amplification, plasma, reported as a radiation toxicity score

	Proprietary test: RadTox™ cfDNA test Lab/Manufacturer: DiaCarta Clinical Lab/DiaCarta Inc
0564T	Oncology, chemotherapeutic drug cytotoxicity assay of cancer stem cells (CSCs), from cultured CSCs and primary tumor cells, categorical drug response reported based on percent of cytotoxicity observed, a minimum of 14 drugs or drug combinations (Reported for ChemoID®)

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

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Revision History

Revision Date	Summary of Changes
09/06/2023	Reviewed and Updated: Updated the background, guidelines and recommendations, and evidence-based scientific references. Literature review did not necessitate any modifications to coverage criteria. The following edits were made for clarity: All CC edited for clarity and consistency.

Intracellular Micronutrient Analysis

Policy Number: AHS – G2099 – Intracellular Micronutrient Analysis	Prior Policy Name and Number, as applicable:
Initial Presentation Date: 09/18/2015 Revision Date: 03/06/2024	

Policy Description

Micronutrients are dietary components, often referred to as vitamins and minerals, which although only required by the body in small amounts, are vital to development, disease prevention, and wellbeing. Micronutrients are not produced in the body and must be derived from the diet (CDC, 2022; Life, 2012). Micronutrients include essential trace elements such as boron, iron, zinc, selenium, manganese, iodine, copper, molybdenum, cobalt, and chromium (Frieden, 1985; WHO, 1973), and essential vitamins such as vitamins A, B, C, D, and K (organic) (Gidden & Shenkin, 2000).

Related Policies

Policy Number	Policy Title
AHS-G2056	Diagnosis Of Idiopathic Environmental Intolerance

Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

1) Intracellular micronutrient panel testing (e.g., SpectraCell, Cell Science Systems cell micronutrient assay, ExaTest) **DOES NOT MEET COVERAGE CRITERIA**.

Table of Terminology

Term	Definition
ASEM	Analytical scanning electron microscopy
CDC	The Centers for Disease Control
CLIA	Clinical Laboratories Improvement Amendments
CLIA '88	Clinical Laboratory Improvement Amendments of 1988
CMA	Cellular micronutrient assay
CMS	Centers for Medicare and Medicaid
CSS	Cell Science Systems
EXA	Energy dispersive x-ray analysis
FDA	Food and Drug Administration
HPLC	High-performance liquid chromatography
LDTs	Laboratory-developed tests
SI	Stimulation index
WHO	World Health Organization

Scientific Background

Micronutrients, such as zinc, selenium, and copper, are involved in metabolic processes, either as catalysts or facilitators for various enzymatic functions. Micronutrient deficiency can result from general malnutrition, a current illness, or side effects of medications or procedures. Nutritional loss may exacerbate severe illness and side effects of medications as the inflammatory response draws micronutrients to the damaged organs, causing an increase in oxidative stress, and normal defense mechanisms to fail (Preiser et al., 2015). For example, oxidative damage in copper deficiency results in muscle weakness and edema, and impaired oxidative status in iodine deficiency leads to a decrease in thyroid hormone synthesis and mental retardation (Pazirandeh, 2024; Pearce et al., 2016).

The measurement of serum vitamin and mineral levels is widely available from numerous commercial testing companies. Normal serum nutrient concentration varies based on its function in the body. Serum concentrations of nutrients involved in regulatory mechanisms, such as calcium and zinc, are maintained within narrow ranges regardless of body stores and any changes only occur with severe nutrient deficiency. Other nutrients, such as carotenoids, vary in the body depending on recent intake or half-life length. Environmental factors, such as infections or stress, can also influence serum nutrient concentrations. Vitamin C, Vitamin B, selenium, and magnesium play a role in reducing the levels of cortisol and adrenalin in the body (McCabe et al., 2017). Nutrient concentrations may also vary based on the tissue. Nutrient concentrations in cell membranes or bone fluctuate less, but these measurements are more difficult to obtain (Elmadfa & Meyer, 2014). Serum nutrient testing is promoted to the public as a nutrient deficiency screening and supplement personalization, but these tests are usually

unwarranted. There is not enough information available regarding the optimal blood levels of vitamins. Moreover, there is a lack of evidence that vitamin supplements prevent disease in healthy adults with low blood levels of vitamins, apart from those with specific diets or conditions. Vitamin deficiencies typically occur in special populations such as the elderly or those with gastric bypass surgery, and not the general public (Fairfield, 2024).

Another possible method of measuring nutrient deficiency is to assess the intracellular concentration (as opposed to the typical serum measurement). Intracellular micronutrient lymphocyte analysis was developed based on the premise that a peripheral blood lymphocyte reflects the genetic and biochemical state of the person at the time it was formed (Shive et al., 1986).

Proprietary Testing

Lymphocyte measurement is the basis of SpectraCell's micronutrient testing procedure. Lymphocytes are isolated from the blood sample and placed in a culture medium containing the optimal levels of nutrients for sustained growth. A given micronutrient is removed, and then growth is measured and compared against the 100% level of growth. For example, Vitamin B6 may be removed from the medium. The growth rate of the cell is theoretically only dependent on vitamin B6 as all other micronutrients are at optimal levels; therefore, any deficiency in cell growth would be caused by issues with intracellular Vitamin B6. This is done for all 31 micronutrients in the panel and results are reported. The micronutrients included in SpectraCell's panels are as follows: Vitamins A, B1, B2, B3, B6, B12, C, D, E, and K, as well as biotin, folate, pantothenate, calcium, magnesium, manganese, zinc, copper, asparagine, glutamine, serine, oleic acid, alpha-lipoic acid, coenzyme Q10, cysteine, glutathione, selenium, chromium, choline, inositol, and carnitine. SpectraCell also provides an assessment of "Total Antioxidant Function," an "Immune Response Score," and measures of fructose sensitivity and glucose-insulin metabolism (SpectraCell, 2021).

Another test analyzing intracellular concentration is ExaTest by IntraCellular Diagnostics. From their laboratory website, this test uses "rapidly metabolizing sublingual epithelial cells under Analytical Scanning Electron Microscopy, (ASEM) an Energy Dispersive X-Ray Analysis, (Exatest) to reflect fast tissue changes of vital mineral electrolytes." This test is primarily for aid with the management of heart disease and provides tissue evaluations of magnesium, sodium, calcium, phosphorus, potassium, and chloride. ExaTest proclaims its ability to follow a patient's metabolic status and assess electrolyte imbalance easily. First, the buccal, epithelial cells are swabbed from the patient. Then the sample is analyzed by the proprietary energy dispersive x-ray analysis and bombarded with X-Rays. Energy is released by wavelengths (unique to each element), and the element composition is analyzed and reported. ExaTest states that the serum or urine of some minerals do not correlate with intracellular levels and that these deficiencies are common in patients with various health issues, particularly heart disease. Buccal cells are used as they are easily accessible and have an easily analyzed structure for electrolytes (Exatest, 2014).

Vibrant America has also developed a test that gives both extracellular and intracellular information on approximately 40 vitamins, minerals, amino acids, fatty acids and antioxidants in the body (Vibrant, 2017). Vibrant America states that the benefits of intracellular testing include the identification of potential functional deficiencies in the cellular nutrient absorption process (which may increase the risk of certain diseases), and the identification of an individual's nutritional status in the previous four to six months (Vibrant, 2017).

Another possible method of analyzing nutrient deficiency is by measuring lymphocyte proliferation in response to micronutrient concentration. Cell Science Systems (CSS) released a cellular micronutrient

assay (CMA) which measures the effect of micronutrients on lymphocyte proliferation when stimulated with a mitogen. According to their protocol, lymphocytes are primarily separated from the patient's whole blood and the patient's own serum is added back to the lymphocytes. The cells are stimulated with a mitogen and baseline lymphocyte proliferation rates (without the addition of micronutrients) are recorded. Next, micronutrients are added to the lymphocyte culture and proliferation rates are compared to the baseline rate. If the addition of micronutrients to the lymphocyte culture enhances lymphocyte proliferation, a nutrient insufficiency is reported. If the lymphocyte proliferation rate with the addition of micronutrients does not exceed the baseline rate, it likely indicates sufficient stores of that nutrient. The CMA measures vitamins, amino acids, minerals, and other nutrients such as carnitine, alpha-ketoglutarate, choline, glutathione, and inositol. By measuring intracellular levels of micronutrients, the test is intended to provide insight into the long-term nutritional status (6 months) versus the short term variability of serum nutrient levels, which is prone to daily fluctuations (Systems, 2022). In 2020, the FDA approved the Baze blood testing kit for at-home use to assess nutrient status by analyzing 10 micronutrients. Through a small sample of whole blood, the testing device determines levels of choline, chromium, copper, magnesium, omega-3, selenium, vitamins B12, D, E, and zinc. The sample is mailed to a certified laboratory and analyzed using mass-spectrometry (Baze, 2020).

Genova Diagnostics released NutrEval FMV® a comprehensive blood and urine test that evaluates over 125 biomarkers and 40 antioxidants, vitamins, minerals, essential fatty acids, and amino acids in patients 2 years and older. These levels provide insight into digestive function, toxic exposure, mitochondrial function, and oxidative stress. According to their website, NutrEval is not meant to be a substitute for other conventional nutritional panels (complete blood count, comprehensive metabolic panel), but rather a complement by providing additional information (Genova Diagnostics, 2021).

Analytical Validity

In a randomized observational analysis, the Cell Science Systems (CSS) cellular micronutrient assay (CMA) was used to examine nutritional status in 845 American individuals aged 13 years and older. Results were expressed as the stimulation index (SI), which is the percentage of lymphocyte stimulation in response to the mitogen. All subjects were divided into two groups based on their diet. The first group had a healthy diet, consisting of whole fresh foods including fruits, vegetables, nuts, while the poor diet group reported high consumption of sweets, fried, frozen, and starchy foods. CMA analysis indicated that the "mean values for micronutrient deficiency were significantly higher in the poor diet group as compared to the healthy diet group with p-values of 0.0017 and 0.0395, respectively." According to the authors, "the adequate functioning of this defensive system is critically impacted by intracellular nutritional status, and its interaction with the host' cells. Lacking adequate nutrition, the immune system is clearly deprived of the components needed to generate an effective immune response" (Steele et al., 2020).

Clinical Utility and Validity

While limited research has been completed regarding intracellular micronutrient lymphocyte analysis, Yamada et al. (2004) did complete a study with 41 type 2 diabetes patients and 50 healthy controls. No participants were taking vitamin supplements at the time of the study. Blood samples were taken from all participants during a fasting state; the researchers determined that the lymphocyte vitamin C level was significantly lower in the type 2 diabetes patients than in controls (Yamada et al., 2004). This study may support the above theory that lymphocytes can be used as an indicator of an individual's nutrient state.

Houston (2010) published a small study stating that treating the intracellular micronutrient deficiencies in combination with optimal diet, exercise and other weight management resulted in reaching blood pressure goals for 62% of a hypertensive population (Houston, 2010). Another small study of 10 patients found that both genders showed overall improvement in their vitamin and mineral cellular storage balance after being tested with SpectraCell's assessment (Frye, 2010). However, the authors of each of the aforementioned studies (Houston, Bucci, Frye, and Shive) are associated with SpectraCell Laboratories. SpectraCell has listed several studies on their website discussing serum versus intracellular deficiencies; from discussing the effect of the inflammatory response on serum micronutrient levels to Vitamin B12's difficult serum profile to micronutrient deficiencies in special populations (SpectraCell, 2024). However, none of these studies reported use SpectraCell's actual method as of 2018, nor did the studies cover the healthy population for which the test is marketed. Most of these studies listed used other methods such as HPLC to measure micronutrient levels instead of the proprietary method provided by SpectraCell. Few other studies listed on SpectraCell's website used lymphocytes as the analyte as well.

In an observational study, Coelho et al. (2020) studied the association between serum and dietary antioxidant micronutrients and advanced liver fibrosis in non-alcoholic fatty liver disease (NAFLD). 72 NAFLD patients were evaluated for levels of retinol, alpha-tocopherol, ascorbic acid, beta-carotene, serum zinc, and selenium. "A high proportion of inadequate serum retinol (20.8%), vitamin C (27%), and selenium (73.6%) was observed in the patients with NAFLD, in addition to a significant inadequacy of vitamin A (98.3%) and vitamin E (100%) intake." Those with advanced liver fibrosis had reduced levels of serum retinol. Overall, "NAFLD patients showed an important serum deficiency and insufficient dietary intake of the evaluated micronutrients" (Coelho et al., 2020).

Guidelines and Recommendations

No studies evaluating the accuracy or clinical utility of intracellular micronutrient testing compared to standard testing for vitamin or mineral levels were identified. In addition, no controlled studies that evaluated changes to patient management or health impact of intracellular micronutrient testing were identified. Limited data are available on correlations between serum and intracellular micronutrient levels. Intracellular micronutrient analysis was not included in reviews on micronutrient analysis (Elmadfa & Meyer, 2014; Raghavan et al., 2016).

No recommendations or practice guidelines recommending intracellular micronutrient testing were identified in a literature search.

Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: http://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Intracellular micronutrient testing is offered by companies SpectraCell, IntraCellular Diagnostics, and Cell

Science Systems Corporation which have Clinical Laboratories Improvement Amendments (CLIA) accredited laboratories. SpectraCell's micronutrient panel test, the IntraCellular Diagnostics ExaTest, Genova Diagnostic's NutrEval FMV, and the Cell Science Systems Cellular Micronutrient Assay (CMA) have not been through the FDA approval process.

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
82128	Amino acids; multiple, qualitative, each specimen
82136	Amino acids, 2 to 5 amino acids, quantitative, each specimen
82180	Ascorbic acid (vitamin c), blood
82310	Calcium; total
82379	Carnitine (total and free), quantitative each specimen
82495	Chromium
82525	Copper
82607	Cyanocobalamin (Vitamin B-12);
82652	Vitamin D; 1, 25 dihydroxy, includes fraction(s), if performed
82725	Fatty acids, nonesterified
82746	Folic acid; serum
82978	Glutathione
83735	Magnesium
83785	Manganese
84207	Pyridoxal phosphate (vitamin b-6)
84252	Riboflavin (vitamin b-2)
84255	Selenium
84425	Thiamine (vitamin b-1)
84446	Tocopherol alpha (Vitamin E)
84590	Vitamin A
84591	Vitamin, not otherwise specified
84597	Vitamin K
84630	Zinc
84999	Unlisted chemistry procedure
86353	Lymphocyte transformation, mitogen (phytomitogen) or antigen induced blastogenesis
88348	Electron microscopy, diagnostic

Procedure codes appearing in policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

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Revision History

Revision Date	Summary of Changes
03/06/2024	Reviewed and Updated: Updated the background, guidelines and recommendations, and evidence-based scientific references. Literature review did not necessitate any modifications to coverage criteria.
03/01/2023	Reviewed and Updated: Updated the background, guidelines and recommendations, and evidence-based scientific references. Literature review did not necessitate any modifications to coverage criteria. The following edits were made for clarity: CC1 edited for clarity and consistency.
03/09/2022	Annual Review: Updated background, guidelines and recommendations, and evidence-based scientific references. Literature review did not necessitate modification to coverage criteria
03/03/2021	Annual review: Updated background and evidence-based scientific references. Literature review did necessitate changes to the CC. Literature review necessitated one change to CC, which was the addition of the "Cell Science Systems cell micronutrient assay" to the following CC: "Intracellular micronutrient panel testing, including but not limited to SpectraCell, Cell Science Systems cell micronutrient assay and ExaTest, DOES NOT MEET COVERAGE CRITERIA."
03/10/2020	Annual review: Updated background and evidence-based scientific references. No guidelines or recommendations were found on this topic. Changed E&I to DNMCC with the preceding statement regarding lack of published scientific literature. Added the wording "including but not limited to SpectraCell and ExaTest" to the CC. Literature review did not necessitate any additional modifications of the coverage criteria.

03/01/2019	Annual review: Background and Evidence-based Scientific References were updated. Literature review did not necessitate any changes to the CC.
09/19/2018	Off-Cycle Review: Added CPT 82136, 82607, 82652, 82746, and 83785.
07/11/2018	Off Cycle Review: Changed CPT code 84999 to PA required
03/16/2018	Off-Cycle Review: Policy was reviewed to change the Annual Review Cycle. Literature review did not necessitate any modification to coverage criteria. No changes in coding.
09/28/2017	Annual review: Background, Definitions, Guidelines and Recommendations, Evidence-based Scientific References were updated. Literature review did not necessitate change in CC.
09/19/2016	Annual review: Literature review did not necessitate any change in coverage criteria.
09/18/2015	Initial presentation

Laboratory Procedures Reimbursement Policy (AHS-R2162)

Policy Number: AHS – R2162 – Avalon Laboratory Procedures Reimbursement Policy	Prior Policy Name and Number, as applicable:
Initial Presentation Date: 12/05/2018 Effective Date: 4/1/2025	

Policy Scope

To be considered for reimbursement, all outpatient laboratory claims should be submitted in accordance with:

- AMA CPT and HCPCS coding and ICD-10 diagnosis coding guidelines
- Other laboratory and pathology coding guidelines
- All applicable regulatory guidelines

This policy outlines additional requirements beyond the guidelines listed above that are required for reimbursement. Note that these guidelines are reviewed and updated periodically.

Modifier Guidelines/Instructions

Technical, Professional, and Global services (-TC, -26 modifiers)

- Before using the -26 or -TC modifiers, verify that these modifiers are allowable with the procedure code.
- Do not append these modifiers to the procedure code when performing the global service.

Tests performed by a Reference laboratory

- When performed by a party other than the treating physician, reporting physician, or other qualified health care professional, the laboratory procedure must be identified by adding modifier -90 to the claim line.
- Only independent clinical laboratories may append modifier -90 to indicate that the service was referred to an outside laboratory.

Repeat Testing

- While treating a patient, it may be necessary to repeat the same laboratory test on the same day to obtain subsequent (multiple) test results. Under these circumstances, the laboratory test performed can be identified by its usual procedure number and the addition of modifier -91.
- Modifier 91 may not be used when tests are rerun to confirm initial results; due to testing problems with specimens or equipment; or for any other reason when a normal, one-time, reportable result is all that is required.

- Modifier 91 may not be used when other code(s) describe a series of test results (e.g., glucose tolerance tests, evocative/suppression testing).

Clinical Laboratory Improvement Amendments (CLIA) Waived Testing

- Laboratory tests which are CLIA-waived must have the QW modifier appended to the procedure code.

Place of Service Guidelines

In accordance with S611b of OBRA of 1989, a referring lab can bill for tests performed by a reference lab only if it meets one of the following exceptions:

- The referring laboratory is in or is part of a rural hospital
- The referring lab and the reference lab are 'subsidiary related.' That is:
  - The referring lab is a wholly owned subsidiary of the reference lab
  - The referring lab wholly owns the reference lab
  - Both the referring lab and reference lab are wholly owned subsidiaries of the same entity.

Non-Reimbursable CPT/HCPCS Codes

Some procedure codes will not be reimbursed due to their expiration or replacement with more appropriate codes.

- AMA drug assay codes 80320 to 80377 are not accepted and will not be reimbursed. Refer to policy T2015, Opioids Testing in Pain Management and Substance Abuse, for guidelines for submitting G0480 to G0483.
- PLA codes will not be reimbursed unless a laboratory policy specifically covers the PLA code.

Edit Types

Outpatient lab claims are consistently evaluated for reimbursement against several standard edit types using administrative information (e.g., claim information, historical claims). The specific edits are described below.

Additional Tests on the Date of Service

The presence or absence of additional tests on a single date of service (DOS) may trigger a reimbursement denial for a claim line.

The exclusivity edit is based upon:

- A list of tests where Correct Coding Initiative (CCI) and/or AMA coding guidance identify that two procedure codes for the test are not permitted for the same patient at the same time because it is only appropriate to charge for one of those procedures.
- Clinical guidelines for testing preclude the simultaneous performing of two tests. For example, individual components of panel procedures codes will not be separately reimbursed when billed with the panel procedure code.
- Technically complex procedures which incorporate simple procedures will not be reimbursed for the same patient on the same DOS. For example, billing for multiple testing methodologies (e.g., direct, amplification, and quantitative testing) for the same microorganism codes is not reimbursed.

Thus, a denial based upon this edit is one that is based upon evaluation of universal, objective criteria related to how the test is being billed, not an assessment of a patient's condition to determine whether both codes were appropriate.

Date of Service

In general, the date of service (DOS) for clinical diagnostic laboratory tests is the date of specimen collection unless the physician orders the test at least 14 days following the patient's discharge from the hospital. When the "14-day rule" applies, the DOS is the date the test is performed, instead of the date of specimen collection.

In the CY 2018 Hospital Outpatient Prospective Payment System (OPPS)/Ambulatory Surgical Center (ASC) final rule published December 14, 2017, CMS established another exception to laboratory DOS policy for Advanced Diagnostic Laboratory Tests (ADLTs) and molecular pathology tests excluded from OPPS packaging policy so that the DOS is the date the test was performed if certain conditions are met. Specifically, in the case of a molecular pathology test or an ADLT that meets the criteria of section 1834A(d)(5)(A) of the Social Security Act, the date of service must be the date the test was performed only if the following conditions are met:

1. The test is performed following a hospital outpatient's discharge from the hospital outpatient department;
2. The specimen was collected from a hospital outpatient during an encounter (as both are defined 42 CFR 410.2);

3. It was medically appropriate to have collected the sample from the hospital outpatient during the hospital outpatient encounter;
4. The results of the test do not guide treatment provided during the hospital outpatient encounter; and
5. The test was reasonable and medically necessary for the treatment of an illness.

Incorrect Diagnosis Code

Select diagnosis and procedure code combinations are permitted or precluded depending on the nature of the policy.

The edit functions to identify those tests that are never appropriate unless the physician has first concluded that the patient presents with the indicated diagnosis. Although the edit is contingent upon the diagnosis of the individual patient, it is not conducting any clinical evaluation of whether the condition, in fact, exists. Rather, the inherent nature of the test (only being indicated for patients with the condition or contraindicated for the condition) and the question of whether the pre-requisite condition is present are the conditions for reimbursement.

Incorrect Patient Age

This edit addresses medical policies with coverage criteria, CPT/HCPCS codes, and diagnosis codes that not are reimbursable based on the patient's age on the DOS.

For example, testing on newborns must be associated with a member who is 28 days of age or younger.

Incorrect Place of Service

This edit is invoked when the Place of Service is identified as inappropriate with the laboratory test/service performed submitted on the claim.

Once per Lifetime Tests

This edit limits the frequency of applicable laboratory services/procedure codes to once in the patient's lifetime.

Certain laboratory services should only be performed once in a patient's lifetime as outlined in medical policy. If a once-per-lifetime test is submitted for reimbursement more than once, the subsequent submissions will not be reimbursed.

Pathologist and Physician Laboratory Providers

If a pathologist and another physician or other qualified health care professional's offices submit identical laboratory codes for the same patient on the same date of service, only the pathologist's service is reimbursable

Incomplete Laboratory Panels

MVP does not routinely compensate for the following, as additional laboratory components of a panel are included in the price of the laboratory panel code itself.

Basic metabolic panel

- More than two basic metabolic panel procedure codes when submitted on the same date of service
- More than one of the following procedure codes (82040, 82247, 84075, 84460, 84450, 84155) when billed with a basic metabolic panel procedure code on the same date of service

Comprehensive metabolic panel

- More than three comprehensive metabolic panel procedure codes when submitted on the same date of service

Electrolyte panel

- More than two electrolyte panel procedure codes when submitted on the same date of service

Hepatic function panel

- More than two hepatic function panel procedure codes when submitted on the same date of service

Renal function panel

- More than three renal function panel procedure codes when submitted on the same date of service

Specimen Validity Test

MVP does not reimburse for specimen validity testing. The following code will deny the same day as drug testing unless modifier 59 is submitted to indicate that the testing is not being performed for specimen validity. The records must also support that the urinalysis performed was not for specimen validity testing and the modifier was appropriately reported.

Code denied 81099, 82570, & 83986

Unit Threshold Met (Daily and Historical)

These edits are invoked when the number of units billed for the procedure on a single DOS or over a period of time exceed an allowed reimbursement quantity without considering any aspect of an individual's specific condition. Maximum units of service are determined by one or more of the following:

- The CPT or HCPCS code description defines the number of units per patient per DOS for a unique billing event.
- Laboratory Coverage Guidelines outlined in medical policy establish the number of units for a laboratory service.
- The service is anatomically or clinically limited to the number of procedures that may be performed and therefore units billed.
- Scientific or statistical analyses demonstrate a reasonable limitation of the number of units that should be performed within a specified period of time.
- Third-parties such as Correct Coding Initiative or Centers for Medicare and Medicaid Services limit reimbursement to a specified number of units.

If a procedure code that is assigned a maximum unit value is reported with a greater unit count, the claim line will be reimbursed only for the number of units up to but not exceeding the allowed maximum.

Sexually Transmitted Disease Testing

The following Sexually Transmitted Disease Testing CPT Codes are relevant to MVP's policy:

CPT Code	Description
87491	Infectious Agent Antigen Detection, Chlamydia
87591	Infectious Agent Antigen Detection, Gonorrhea
87661	Infectious Agent Antigen Detection, Trichomonas Vaginalis
87801	Infectious Agent Antigen Detection, Multiple Organisms

MVP will reimburse the more comprehensive multiple organism code when any two or more of the single test codes (87491, 87591, and/or 87661) are billed separately for the same provider and the same date of service.

Regardless of the number of units billed for a single code, reimbursement will be made based a single unit of the comprehensive code 87801. This policy applies across all lines of business.

MVP covers the full cost of Sexually Transmitted Disease testing with no co-pays, deductibles, or co-insurance for Members in accordance with state and federal regulations when these services are the primary reason for a visit. Providers should still bill MVP for these services as appropriate; however, no co-pay/co-insurance/cost share should be taken at the time of service. Claims will still be subject to clinical edits and bundling. Providers should check the Member's benefits to determine if preventive services apply to their plan.

Non-Covered Services

- Laboratory and pathology services that are rendered in conjunction with an inpatient stay or an observation stay. (They are included in the respective global payment; for example, DRG, per diem, etc.)
- Handling charges
- Specimen collection
- Routine venipuncture charges made in conjunction with blood or related laboratory services or evaluation and management services
- Paternity blood tests
- NAbFeron (IFNb) antibody test

- Mandated drug testing (e.g., court-ordered, residential monitoring, non-medically necessary testing)
- Laboratory and pathology services submitted with unlisted CPT codes when an appropriate specific code is available
- Laboratory and pathology services provided at no charge by state agencies, including but not limited to pertussis and rubella
- Drugs, devices, treatments, procedures, laboratory and pathology tests that are experimental, unproven, or investigational and not supported by evidence-based medicine and established peer reviewed scientific data
- Employment drug screening
- NAB (neutralizing antibody testing) in multiple sclerosis patients
- Lipoprotein subclass testing in the evaluation of cardiovascular disease
- Quantitative urine drug testing where there has been no underlying qualitative test or where the qualitative test is negative

References

1. Centers for Medicare and Medicaid Services, "Medically Unlikely Edits" https://www.cms.gov/Medicare/Coding/NationalCorrectCodInitEd/MUE.html
2. American Medical Association, Current Procedural Terminology (CPT ®), Professional Edition
3. https://www.cms.gov/Regulations-and-Guidance/Guidance/Manuals/downloads/clm104c16.pdf
4. CMS Pub. 100-04, chapter 16, section 40.1.1 external link (PDF, 497 KB)
5. https://www.cms.gov/files/document/medicaid-ncci-policy-manual-2022-chapter-10.pdf

Revision History

Review Date	Summary of Changes
06/19/2024	Updated section IV., was "IV. Genetic Counseling Reimbursement Guidelines", is now "IV. Genetic Counseling Considerations". Updated language to reflect that Avalon does not adjudicate genetic counseling and thus it does not "meet coverage criteria". However, some genetic tests may require genetic counseling. Language in section IV was adjusted to reflect this. In section VI, a genetic panel was defined and coding considerations were added and/or adjusted. Added new bullet point 2: "• Multi-gene panels must contain the genes specified in the AMA CPT coding description." Former bullet 2, now bullet 3, updated. Previously read: "•If there is not a specific next generation sequencing (NGS) procedure code that represents the requested test, the procedure may be represented by a maximum of ONE unit of 81479 [unlisted molecular pathology procedure] (i.e., 81479 X 1 should account for all

	remaining gene testing) OR all genes tested on the panel must be represented by ALL appropriate Molecular Pathology Tier 1 or 2 procedure codes (with exception of 81479 x 1 only being listed once if it appropriately represents more than one gene in the panel)." Now reads: "• If there is not a specific next generation sequencing (NGS) procedure code that represents the requested test, a maximum of ONE unit of 81479 [unlisted molecular pathology procedure] may be billed." Former bullet 4, now bullet 5, updated. Previously read: "• If ALL codes that represent the testing of the panel are not submitted, the test will be denied as not medically necessary due to incorrect coding process, as neither laboratory nor clinical reviewer should assign meaning to incomplete unspecified panel codes." Now reads: "• If incorrect codes are submitted to represent panel testing, ALL codes submitted will be denied as not medically necessary due to incorrect coding process."

Laboratory Testing for the Diagnosis of Inflammatory Bowel Disease

Policy Number: AHS – G2121 – Laboratory Testing for the Diagnosis of Inflammatory Bowel Disease	Prior Policy Name and Number, as applicable: • G2121 – Serum Antibodies for the Diagnosis of Inflammatory Bowel Disease
Initial Presentation Date: 09/18/2015 Revision Date: February 1, 2025	

Policy Description

Inflammatory bowel disease (IBD) is a class of inflammatory bowel disorders comprised of two major disorders: ulcerative colitis and Crohn's disease each with distinct pathologic and clinical characteristics (Peppercorn & Cheifetz, 2023).

Ulcerative colitis (UC) is a chronic inflammatory condition characterized by relapsing and remitting episodes of inflammation limited to the mucosal layer of the colon (Silverberg et al., 2005) beginning at the rectum and may extend in a proximal and continuous fashion to involve other parts of the colon (Peppercorn & Kane, 2023).

Crohn's disease (CD) is characterized by patchy transmural inflammation (skip lesions) of the gastrointestinal tract resulting in sinus tracts, and ultimately microperforations and fistulae (Silverberg et al., 2005). It may also lead to fibrosis, strictures and to obstructive clinical presentations that are not typically seen in ulcerative colitis (Gasche et al., 2000; Peppercorn & Kane, 2022).

Related Policies

Policy Number	Policy Title
AHS-G2043	Celiac Disease Testing

AHS-G2060	Fecal Analysis in The Diagnosis of Intestinal Dysbiosis
AHS-G2061	Fecal Calprotectin Testing in Adults
AHS-G2155	General Inflammation Testing

Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

1) For the workup and monitoring of individuals with inflammatory bowel disease (IBD), the use of serologic markers (e.g., anti-neutrophil cytoplasmic antibody [ANCA]; perinuclear ANCA; anti-*Saccharomyces cerevisiae* antibody; antibody to Escherichia coli outer membrane porin C; anti-CBir1 flagellin antibody; antibody to *Pseudomonas fluorescens*-associated sequence I2; antichitobioside, antilaminaribioside, or antimannobioside antibodies; pyruvate kinase M2) **DOES NOT MEET COVERAGE CRITERIA**.

2) For the diagnosis or monitoring of individuals with IBD, the use of diagnostic algorithm-based testing, including testing that combines serologic, genetic, and inflammation markers (e.g., Prometheus® testing), **DOES NOT MEET COVERAGE CRITERIA**.

3) Genetic testing for IBD **DOES NOT MEET COVERAGE CRITERIA**.

Table of Terminology

Term	Definition
7C4	7α-hydroxy-4-cholesten-3-one
AAST	American Association for the Surgery of Trauma
ACCA	Anti-chitobioside carbohydrate antibody
ACG	American College of Gastroenterology
ACP	Antibodies to the Crohn's disease peptide
AGA	American Gastroenterological Association
ALCA	Laminaribioside
ALCA IgG	Antilaminaribioside antibodies
AMCA	Antimannobioside carbohydrate
AMCA IgG	Antimannobioside antibodies
ANCA	Anti-neutrophil cytoplasmic antibody
anti-cBir1	Anti-CBir1 flagellin antibody
anti-CUZD1	CUB and zona pellucida-like domains-containing protein 1
anti-GAB	Anti-goblet cell
anti-GP2	Anti-glycoprotein 2
anti-I2	Antibody to pseudomonas fluorescens-associated sequence I2
anti-LFS	Anti-DNA-bound-lactoferrin
anti-OmpC	Antibody to escherichia coli outer membrane porin C

APA	Anti-pancreatic antibodies
ASCA	Anti-*saccharomyces cerevisiae a*ntibody
ATG16L1	*Autophagy related 16 like 1* gene
AUC	Area under the curve
B2-M	Beta 2-microglobulin
BD	Inflammatory bowel disease
BSG	British Society of Gastroenterology
CD	Crohn's disease
CD	Celiac disease
CGD	Chronic granulomatous disorder
CLIA '88	Clinical Laboratory Improvement Amendments of 1988
CMS	Centers for Medicare and Medicaid Services
CRP	C-reactive protein
DNA	Deoxyribonucleic acid
DOR	Diagnostic odds ratio
ECCO	European Crohn's and Colitis Organisation
ECM1	Extracellular matrix protein 1
ELISA	Enzyme-linked immunoassay
ESGAR	European Society of Gastrointestinal and Abdominal Radiology
ESR	Erythrocyte sedimentation rate
FC	Fecal calprotectin
FDA	Food and Drug Administration
GI	Gastrointestinal
HLH	Hemophagocytic lymphocytic histiocytosis
IBD	Inflammatory bowel disease
IBS	Irritable Bowel Syndrome
ICAM-1	Intercellular Adhesion Molecule 1
IL-10R	Interleukin-10 receptor
LDTs	Laboratory developed tests
mRNA	Messenger ribonucleic acid
NADPH	Reduced nicotinamide adenine dinucleotide phosphate
NASPGHAN	North American Society for Pediatric Gastroenterology, Hepatology, And Nutrition
NICE	National Institute for Health and Care Excellence
NKX2-3	*NK2 homeobox 3* gene
NPV	Negative predictive value
PAB	Pancreatic antibody
pANCA	Perinuclear anti-neutrophilic cytoplasmic antibody
PKM2	Pyruvate kinase M2
PPV	Positive predictive value
PROMs	Patient-reported outcome measures
SAA	Human serum amyloid A
SNPs	Single nucleotide polymorphisms
STAT3	Signal transducer and activator of transcription 3
UC	Ulcerative colitis
VCAM-1	Vascular cell adhesion protein 1
VEGF	Vascular endothelial growth factor

VEO-IBD	Very early onset inflammatory bowel disease
WES	Whole exome sequencing
WGO	World Gastroenterology Organisation
WGS	Whole genome sequencing
WSES	World society of emergency surgery
XIAP	X-linked inhibitor of apoptosis protein

Scientific Background

The diagnoses of Crohn's disease (CD) and ulcerative colitis (UC) depend on a combination of clinical, laboratory, radiographic, endoscopic, and histological criteria. Differential diagnosis can be challenging but is highly important toward treatment and prognosis. Serological markers could be of value in differentiating CD from UC, in cases of indeterminate colitis, and in predicting the disease course of IBD (Peppercorn & Cheifetz, 2023; Peppercorn & Kane, 2022, 2023).

Investigations based on animal models have led to the current theory that chronic intestinal inflammation is the result of an aberrant immunologic response to commensal bacteria within the gut lumen (Blumberg et al., 1999; Strober et al., 2002). Immune responses toward commensal enteric organisms have been investigated in CD and UC (Akasaka et al., 2015; D'Haens et al., 1998). Patients with IBD can have a loss of tolerance to specific bacterial antigens and autoantigens. These distinct antibody response patterns may indicate unique pathophysiological mechanisms in the progression of this complicated disease and may underlie the basis for the development of specific phenotypes (Landers et al., 2002; Peeters et al., 2001).

Numerous serological markers have been proposed as having utility in assessment of IBD patients. The most widely studied markers are the antineutrophil cytoplasmic antibodies (pANCA) and anti-*Saccharomyces cerevisiae* antibodies (ASCA), particularly for diagnosing IBD and distinguishing CD from ulcerative colitis (Higuchi, 2022; Peppercorn & Kane, 2022). pANCA is thought to be an antibody corresponding to histone 1 whereas ASCA is an antibody against mannan from baker's yeast (Mitsuyama et al., 2016). Although there have been promising results regarding the clinical validity of these antibodies (Reese et al., 2006; Ruemmele et al., 1998; Sandborn et al., 2000), its utility in indeterminate bowel disease is uncertain (Joossens et al., 2002; Peeters et al., 2001). ASCA were present in 50 percent of patients with celiac disease and described in cystic fibrosis and intestinal tuberculosis, suggesting that they may reflect a nonspecific immune response in small bowel disease (Condino et al., 2005; Granito et al., 2005).

Additional antibody tests under investigation include laminaribioside (ALCA), chitobioside (ACCA), CBir1 flagellin, OmpC, and I2. ALCA and ACCA are antiglycan antibodies whereas the CBir1 flagellin comes from an indigenous species of bacteria (Dotan et al., 2006; Targan et al., 2005). OmpC is an antibody to an outer membrane protein of *E. coli* and I2 is an antibody against the I2 component of *Pseudomonas fluorescens* (Mitsuyama et al., 2016). The accuracy and predictive value of antibody tests is uncertain (Wang et al., 2017) and the prevalence of these antibodies in patients with a variety of inflammatory diseases affecting the gut has not been well-studied.

Additionally, bile acid deficiency--as indicated by serum 7α-hydroxy-4-cholesten-3-one (7C4) --has been documented in patients with irritable bowel syndrome (IBS) (Donato et al., 2018; Vijayvargiya et al., 2018). This test has shown utility as an alternative test to measuring bile acids in stool (Walters & Pattni, 2010), but it is not recommended in the workup for IBD.

Another proposed biomarker for IBD is serum pyruvate kinase M2 (PKM2), which is "emerging" in IBD as a mediator of inflammatory processes. Almousa et al. (2018) evaluated its association with IBD and its correlation with traditional IBD indices, BD disease type, and intestinal microbiota. The authors found that serum PKM2 levels were 6 times higher in IBD patients compared to healthy controls. However, no sensitivity to disease phenotype or localization of inflammation was observed. A positive correlation between PKM2 and *Bacteroidetes* was identified, as well as a negative correlation between PKM2 and *Actinobacteria*. The investigators concluded that their data "suggests PKM2 as a putative biomarker for IBD and the dysbiosis of microflora in CD," but noted that further validation was required (Almousa et al., 2018).

Genetic studies have identified over 200 distinct susceptibility loci for irritable bowel disease with a significant portion of these overlapping with Crohn's and ulcerative colitis (Jostins et al., 2012; Liu et al., 2015). Most of these are located within introns, which more likely modulate the expression of proteins, with each only conferring a slight increase in risk (Snapper & Abraham, 2022). Altogether, the known loci only explain ~13% of variation in disease liability (Jostins et al., 2012). These results indicate that the genetic architecture of IBD represents that of multifactorial complex traits where a combination of multiple genes, along with the environment, lead to disease (Liu & Anderson, 2014). Given the low predictive value of individual genetic markers and high number of putative risk alleles, genetic testing does not currently offer much in terms of clinical utility (Lichtenstein et al., 2018; Liu & Anderson, 2014; McGovern et al., 2015; Shirts et al., 2012).

Laboratory evidence of inflammation is common in IBD. Fecal calprotectin, lactoferrin, ESR and CRP have each been correlated with disease activity (Lewis, 2011; Menees et al., 2015), but are not specific. Additional inflammatory markers including vascular endothelial growth factor, intercellular adhesion molecule, vascular adhesion molecule, and serum amyloid A offer no significant advantage (Shirts et al., 2012). Fecal calprotectin has been shown to be useful to help differentiate the presence of IBD from irritable bowel syndrome and in monitoring disease activity and response to treatment (Lichtenstein et al., 2018). Inflammation and calprotectin testing are discussed in greater detail in AHS-G2155 and AHS-G2061, respectively.

Clinical Utility and Validity

Panels to improve the predictive value of IBD testing incorporating serologic, genetic, and inflammation markers have been created (Plevy et al., 2013). The clinical validity and utility of antibody tests and panels of combinations of serologic tests for the diagnosis of IBD and the disease course and severity are still uncertain (Benor et al., 2010; Coukos et al., 2012; Kaul et al., 2012; Sura et al., 2014; Wang et al., 2017). For example, Prometheus Biosciences offers a series of tests intended for IBS. This series includes "IBDsgi Diagnostic," which evaluates 17 biomarkers (serological and genetic markers, intended to provide "diagnostic and prognostic clarity,"(Prometheus, 2022a) "Crohn's Prognostic" (evaluates "proprietary serologic (anti-CBir1, anti-OMPC, DNAse sensitive pANCA) and genetic (NOD2 variants SNPs 8,12,13) markers"), and "Monitr" (evaluates 13 biomarkers to provide an "Endoscopic Healing Index Score" which represents endoscopic disease activity) (Prometheus, 2022b). In February 2022, Prometheus announced the release of PredictrPK IFX, a test that helps healthcare providers with biologic dose optimization by using individualized pharmacokinetic modeling. According to the Prometheus site, "PredictrPK IFX combines serology markers, patient-specific variables, current dosing information, and a proprietary machine-learning algorithm to provide individualized actionable insights to optimize the dose and interval for inflammatory bowel disease (IBD) patients treated with infliximab (IFX) or IFX biosimilars" (Prometheus, 2022c).

Mitsuyama et al. (2014) conducted a multicenter study to explore the possible diagnostic utility of antibodies to the CD peptide (ACP) in patients with CD. A total of 196 patients with CD, 210 with UC, 98 with other intestinal conditions, and 183 healthy controls were examined. In CD patients, ACP had a higher sensitivity and specificity (63.3% and 91.0%, respectively) than ASCA (47.4% and 90.4%, respectively). ACP was also found to be negatively associated with disease duration. The authors concluded that "ACP, a newly proposed serologic marker, was significantly associated with CD and was highly diagnostic. Further investigation is needed across multiple populations of patients and ethnic groups, and more importantly, in prospective studies" (Mitsuyama et al., 2014).

Kaul et al. (2012) performed a meta-analysis/systemic review aimed to evaluate the diagnostic value, as well as the association of anti-glycan biomarkers with IBD susceptible gene variants, disease complications, and the need for surgery in IBD. A total of 23 studies were included consisting of 14 in the review and 9 in the meta-analysis. They found that "individually, anti-Saccharomyces cerevisiae antibodies (ASCA) had the highest diagnostic odds ratio (DOR) for differentiating IBD from healthy (DOR 21.1), and CD from UC (DOR 10.2…)" (Kaul et al., 2012). The authors concluded, "ASCA had the highest diagnostic value among individual anti-glycan markers. While anti-chitobioside carbohydrate antibody (ACCA) had the highest association with complications, ASCA and ACCA associated equally with the need for surgery" (Kaul et al., 2012).

Schoepfer et al. (2008) aimed to determine the accuracy of fecal markers, C-reactive protein (CRP), blood leukocytes, and antibody panels for discriminating IBD from IBS. Sixty-four patients with IBD, 30 patients with IBS, and 42 healthy controls were included within the study. They found that "Overall accuracy of tests for discriminating IBD from IBS: IBD-SCAN 90%, PhiCal Test 89%, LEUKO-TEST 78%, Hexagon-OBTI 74%, CRP 73%, blood leukocytes 63%, CD antibodies (ASCA+/pANCA- or ASCA+/pANCA+) 55%, UC antibodies (pANCA+/ASCA-) 49%. ASCA and pANCA had an accuracy of 78% for detecting CD and 75% for detecting UC, respectively. The overall accuracy of IBD-SCAN and PhiCal Test combined with ASCA/pANCA for discriminating IBD from IBS was 92% and 91%, respectively" (Schoepfer et al., 2008).

Plevy et al. (2013) validated a diagnostic panel incorporating 17 markers. The markers were as follows: "8 serological markers (ASCA-IgA, ASCA-IgG, ANCA, pANCA, OmpC, CBir1, A4-Fla2, and FlaX), 4 genetic markers (ATG16L1, NKX2-3, ECM1, and STAT3), and 5 inflammatory markers (CRP, SAA, ICAM-1, VCAM-1, and VEGF)." A total of 572 patients with CD, 328 with UC, 427 non-IBD controls, and 183 controls were assessed. These results were compared to another panel with serological markers only. The extended panel increased the IBD vs non-IBD discrimination area under the curve from 0.80 to 0.87 and the CD vs UC from 0.78 to 0.93. The authors concluded that "incorporating a combination of serological, genetic, and inflammation markers into a diagnostic algorithm improved the accuracy of identifying IBD and differentiating CD from UC versus using serological markers alone" (Plevy et al., 2013).

Biasci et al. (2019) validated a 17-gene prognostic classifier. The classifier was intended to separate IBD patients into two subgroups of prognosis, IBDhi (poorer prognosis) and IBDlo. Two validation cohorts were used, one of CD (n=66) and one of UC (n=57). IBDhi (separated by the classifier) patients experienced both an "earlier need for treatment escalation (hazard ratio=2.65 (CD), 3.12 (UC)) and more escalations over time (for multiple escalations within 18 months: sensitivity=72.7% (CD), 100% (UC); negative predictive value=90.9% (CD), 100% (UC)" (Biasci et al., 2019).

Czub et al. (2014) compared PKM2 to fecal calprotectin (FC) as markers for mucosal inflammation in IBD. A total of 121 patients (75 with UC, 46 with CD) were compared to 35 healthy controls. The authors found that, PKM2 was "inferior" to FC. The differences in the area under curve were as follows: 0.10 (FC

above PKM2, IBD), 0.14 (UC), and 0.03 (IBD). PKM2 was also considered inferior to FC in differentiating patients from mild UC from healthy patients by an AUC of 0.23 (Czub et al., 2014).

Kovacs et al. (2018) investigated "prognostic potential of classic and novel serologic antibodies regarding unfavorable disease course in a prospective ulcerative colitis (UC) patient cohort". They measured the auto-antibodies anti-neutrophil cytoplasmic (ANCA), anti-DNA-bound-lactoferrin (anti-LFS), anti-goblet cell (anti-GAB) and anti-pancreatic (pancreatic antibody (PAB): anti-CUZD1 and anti-GP2) and the anti-microbial antibodies anti-Saccharomyces cerevisiae (ASCA) IgG/IgA and anti-OMP Plus™ IgA. A total of 187 patients were included. The authors found a total of "73.6%, 62.4% and 11.2% of UC patients were positive for IgA/IgG type of atypical perinuclear-ANCA, anti-LFS and anti-GAB, respectively." Occurrences of PABs were 9.6%, ASCA IgA/IgG was 17.6%, and anti-OMP IgA was 19.8%. IgA type PABs were found to be more prevalent in patients with primary sclerosing cholangitis (37.5% vs. 4.7% for anti-CUZD1 and 12.5% vs. 0% for anti-GP2). IgA type ASCA was associated with a higher risk for requiring long-term immunosuppressant therapy. The authors found that none of the autoantibodies, either alone or in combination, were associated with the "risk of development of extensive disease or colectomy," although "multiple antibody positivity [≥3]" was associated with UC-related hospitalization. Overall, the authors concluded that "Even with low prevalence rates, present study gives further evidence to the role of certain antibodies as markers for distinct phenotype and disease outcome in UC. Considering the result of the multivariate analysis the novel antibodies investigated do not seem to be associated with poor clinical outcome in UC, only a classic antibody, IgA subtype ASCA remained an independent predictor of long-term immunosuppressive therapy" (Kovacs et al., 2018).

Ben-Shachar et al. (2019) evaluated the impact of genotype variations on serological biomarkers. The authors examined three *NOD2* variants (1007fs, G908R, R702W) and an *ATG16L1* variant (A300T). Then, the authors analyzed the antiglycan antibodies anti-*Saccharomyces cerevisiae* (ASCA), antilaminaribioside (ALCA), antichitobioside (ACCA), and antimannobioside carbohydrate (AMCA). A total of 308 IBD patients were included, "130 with Crohn's Disease (CD), 67 with ulcerative colitis (UC), 111 with UC and an ileal pouch (UC-pouch), and 74 healthy controls." ACCA was found to be "positive" in 28% of CD patients with the *ATG16L1* A300T variant, compared to only 3% in patients without the variant. ASCA was found to be positive in 86% of patients with the 1007fs variant, compared to 36% without the variant. UC-pouch patients with the 1007fs variant were also found to have "elevated" ASCA and ALCA levels compared to those without (50% vs 7% and 50% vs 8% respectively). The authors also found that the genetic variants were not associated with serologic responses in healthy controls and "unoperated" UC patients. The authors concluded that "Genetic variants may have disease-specific phenotypic (serotypic) effects. This implies that genetic risk factors may also be disease modifiers" (Ben-Shachar et al., 2019).

Ahmed et al. (2019) examined the association between six serological markers and Crohn's Disease (CD) activity. The six markers evaluated were "ASCA-IgA, ASCA-IgG, anti-OmpC IgA, anti-CBir1 IgG, anti-A4Fla2 IgG and anti-FlaX IgG". A total of 135 patients were included. The authors found that CD patients with high anti-Cbir1 IgG at baseline were 2.06 times more likely to have active clinical disease. The other five autoantibodies were not found to have significant impact on clinical course. The authors concluded that "High levels of anti-Cbir1 IgG appear to be associated with a greater likelihood of active CD. Whether routine baseline testing for anti-Cbir1 IgG to predict a more active clinical course is warranted needs more research" (Ahmed et al., 2020; Duarte-Silva et al., 2019).

Eltabbakh (2021) studied the diagnostic utility of beta 2-microglobulin (B2-M) as a biomarker in patients with IBS and UC. B2-M is a protein released by activated T and B lymphocytes and has shown to increase in inflammatory conditions. 40 patients with UC, 20 patients with IBS, and 20 healthy subjects were

enrolled in the study. Overall, there was a higher mean of B2-M values in the UC patients (1.93) than IBS patients (1.51) or healthy subjects (1.43). At a cut off value of >1.5, sensitivity (75%), specificity (70%), PPV (83.3%), NPV (58.3%), and accuracy (0.753%) were measured. It was concluded that "B2-M level may have a diagnostic and differentiating utility between UC cases and IBS-D type as well as a potential indicator of disease activation in UC patients" (Eltabbakh, 2021).

Gao and Zhang (2021) studied the use of serological markers for the diagnosis of Crohn's disease. 196 suspected CD patients were enrolled in the study and ELISA was used to study the expression of various biomarkers including ASCA-IgG, ASCA-IgA, AYMA-IgG, AYCA-IgA, FI2Y-IgG, and pANCA. Overall, ASCA was found to be the most accurate serological marker for the differential diagnosis of CD. It was also noted that a combination of markers resulted in a higher sensitivity and NPV. There was no relation noted between the expression of ASCA and disease behavior at diagnosis (Gao & Zhang, 2021).

Nakov et al. (2022) performed a review of current studies related to IBS and IBD biomarker diagnosis and management, including how to distinguish IBS from IBD (as a note, IBS is a disorder of the gastrointestinal tract while IBD constitutes inflammation or destruction of the bowel wall. Crohn's disease and ulcerative colitis fall under an IBD etiology). The authors focused on the most clinically validated biomarkers to-date and summarized the biological rationale, diagnostic, and clinical value. The authors wrote, "there are well-established serological markers that help differentiate IBS from IBD. These include ASCA, which facilitates the differential diagnosis of Crohn's disease (CD) and ulcerative colitis (UC), predominantly in the disease's early stages. The serum concentration of ASCA is considerably higher in patients with CD than in those with UC. Thus, ASCA can be employed in differentiating organic disease from IBS." They also noted "the other autoantibodies that can be used in distinguishing IBS from IBD are the anti-neutrophil cytoplasmic antibody. They target antigens present in neutrophils and are positive in 50–80% of the UC patients"(Nakov et al., 2022).

Reese et al. (2006) performed a meta-analysis of dozens of studies to assess the diagnostic precision of ASCA and pANCA in pinpointing irritable bowel disease, as well as the role of these particular serum antibodies in differentiating Crohn's from ulcerative colitis. Using 60 different studies, comprising 3,841 UC and 4,019 CD patients, they calculated sensitivity, specificity, and likelihood ratio for different test combinations. The ASCA+ with PANCA- test had the highest sensitivity for Crohn's disease at 54.6%; the specificity was 92.8%. The sensitivity and specificity of pANCA+ tests for ulcerative colitis were 55.3% and 88.5%, respectively. Sensitivity and specificity or pANCA+ were improved in a pediatric subgroup when combined with an ASCA test. In the pediatric cohort, sensitivity was 70.3% and specificity was 93.4%. In conclusion, the authors write that "ASCA and pANCA testing are specific but not sensitive for CD and UC, but that it may be particularly useful for differentiating between CD and UC in the pediatric population" (Reese et al., 2006).

Guidelines and Recommendations

American Gastroenterological Association (AGA)

No guideline or position statement from AGA on specific use of immunologic or genetic markers for the diagnosis of inflammatory bowel disease was found. The AGA assessment algorithms used for both Crohn's disease and ulcerative colitis do not include genetic testing or combinatorial serologic-genetic testing approaches, such as the Prometheus® testing methodology (AGA, 2015).

In 2021, the AGA published a guideline on the medical management of severe luminal and perianal fistulizing Crohn's disease (Feuerstein et al., 2021). While the guideline focuses on therapeutic approaches (i.e., different drug classes for Crohn's disease), it does make a statement on perceived

future research needs and evidence gaps. AGA notes: "There remains an urgent need for improved patient-specific predictors, clinical and biologic, of response and harm to a particular drug or drug class to improve the rational choice of initial and second-line therapeutic agents in a given patient. The need is especially great in special populations, such as those with fistulizing disease or aggressive and recurrent fibrostenosing disease. Overall, the data on risk-stratifying individual patients into low and high risk of disease complications and disability remain poor"(Feuerstein et al., 2021).

Regarding the laboratory evaluation of functional diarrhea and diarrhea-predominant irritable bowel syndrome in adults (IBS-D), AGA recommends the following:

> "1. In patients presenting with chronic diarrhea, AGA suggests the use of either fecal calprotectin or fecal lactoferrin to screen for inflammatory bowel disease (IBD).
>
> 2. In patients presenting with chronic diarrhea, AGA suggests against the use of erythrocyte sedimentation rate or C-reactive protein to screen for IBD.
>
> 3. In patients presenting with chronic diarrhea, AGA recommends testing for Giardia.
>
> 4. In patients presenting with chronic diarrhea with no travel history to or recent immigration from high-risk areas, AGA suggests against testing for ova and parasites (other than Giardia).
>
> 5. In patients presenting with chronic diarrhea, AGA recommends testing for celiac disease with immunoglobulin A (IgA) tissue transglutaminase and a second test to detect celiac disease in the setting of IgA deficiency.
>
> 6. In patients presenting with chronic diarrhea, AGA suggests testing for bile acid diarrhea.
>
> 7. In patients presenting with chronic diarrhea, AGA makes no recommendation for the use of currently available serologic tests for diagnosis of irritable bowel syndrome (IBS)" (Smalley et al., 2019).

A 2021 clinical practice guideline from AGA recommends the below as best practice advice for the diagnosis of IBD in elderly patients:

> "1. A diagnosis of inflammatory bowel disease (IBD) (Crohn's disease, ulcerative colitis) should be considered in older patients who present with diarrhea, rectal bleeding, urgency, abdominal pain or weight loss because up to 15% of new diagnoses of IBD occur in individuals older than 60 years.
>
> 2. Fecal calprotectin or lactoferrin may help prioritize patients with a low probability of IBD for endoscopic evaluation. Individuals presenting with hematochezia or chronic diarrhea with intermediate to high suspicion for underlying IBD, microscopic colitis or colorectal neoplasia should undergo colonoscopy.
>
> 3. In elderly patients with segmental left-sided colitis in the setting of diverticulosis, consider a diagnosis of segmental colitis associated with diverticulosis in addition to the possibility of Crohn's disease or IBD-unclassified" (Ananthakrishnan et al., 2021).

American College of Gastroenterology (ACG)

The ACG published guidelines (Lichtenstein et al., 2018) on the management of Crohn's disease which state:

- "The diagnosis of Crohn's disease (CD) is based on a combination of clinical presentation and endoscopic, radiologic, histologic, and pathologic findings that demonstrate some degree of focal, asymmetric, and transmural granulomatous inflammation of the luminal GI tract. Laboratory testing is complementary in assessing disease severity and complications of disease. There is no single laboratory test that can make an unequivocal diagnosis of CD. The sequence of testing is dependent on presenting clinical features."
- "Initial laboratory investigation should include evaluation for inflammation, anemia, dehydration, and malnutrition."
- "Genetic testing is not indicated to establish the diagnosis of Crohn's disease."
- "Routine use of serologic markers of IBD to establish the diagnosis of Crohn's disease is not indicated."

The ACG guidelines on Ulcerative Colitis in adults (Rubin et al., 2019) state:

- "We recommend against serologic antibody testing to establish or rule out a diagnosis of UC (strong recommendation, very low quality of evidence)."
- "We recommend against serologic antibody testing to determine the prognosis of UC (strong recommendation, very low quality of evidence)."
- The ACG also mentions perinuclear antineutrophil cytoplasmic antibodies (pANCAs) as a proposed serological marker, but they observe that "there is currently no role for such testing to determine the likelihood of disease evolution and prognosis" and that the marker has low sensitivity for diagnostic purposes.
- Overall, "the yield of genetic or serologic markers in predicting severity and course of UC has been modest at best, and their use cannot be recommended in routine clinical practice based on available data" (Rubin et al., 2019).

The ACG released guidelines on management of IBS in adults. The recommendations state:

- "We recommend that serologic testing be performed to rule out celiac disease (CD) in patients with IBS and diarrhea symptoms.
- We suggest that either fecal calprotectin or fecal lactoferrin and C-reactive protein be checked in patients without alarm features and with suspected IBS and diarrhea symptoms to rule out inflammatory bowel disease.
- We recommend against routine stool testing for enteric pathogens in all patients with IBS" (Lacy et al., 2021).

European Crohn's and Colitis Organisation (ECCO)

ECCO states that the Montréal classification of CD is advocated. Therefore, "genetic tests or serological markers should currently not be used to classify CD in clinical practice" (Gomollón et al., 2016).

In a 2017 update for UC, ECCO states that "the routine clinical use of genetic or serological molecular markers is not recommended for the classification of ulcerative colitis." ECCO also notes that the most widely studied marker is the pANCAs, but they have "limited sensitivity" and "their routine use for the diagnosis of UC and for therapeutic decisions is not clinically justified" (Magro et al., 2017).

ECCO also published a "harmonization of the approach to Ulcerative Colitis Histopathology." A section titled "Correlation of Histological Scores with Biomarkers" is included. However, only fecal biomarkers (such as fecal lactoferrin and calprotectin) are mentioned, with no mention of serological biomarkers (Magro et al., 2020).

ECCO also published the "ECCO Guidelines on Therapeutics in Crohn's Disease: Medical Treatment." While the guideline mainly focused on therapeutic agents, it does advocate for identification of important biomarkers to biologic effect. ECCO writes, "there is a clear need to identify biomarkers that could guide therapeutic choices, and to conduct appropriately sized head-to-head trials that could allow for the identification of patient subgroups who would benefit from a given biologic over the other" (Torres et al., 2019).

ECCO expounds on their guidelines for the prevention, diagnosis, and management of infections in inflammatory bowel disease in a series of statements. A list of the relevant guidance is captured below.

- "Serological screening for hepatitis A, B, C, HIV, Epstein-Barr virus, cytomegalovirus, varicella zoster virus, and measles virus [in the absence of documented past infection or vaccination for the latter two] is recommended for all IBD patients at baseline [EL4] and especially before or during immunosuppressive treatment [EL1]. A Pap smear for human papillomavirus screening is also recommended [EL1]"

- "Immunohistochemistry [IHC], possibly tissue polymerase chain reaction [PCR], or both, are essential for confirming active CMV infection [colitis] in IBD and should be the standard tests [EL2]. Findings and potential interventions should be discussed in the clinical context"

- "Immunosuppressed female IBD patients should undergo annual cervical cancer screening [EL3]"

- "Routine prophylactic HPV vaccination is recommended for both young female and young male patients with IBD [EL2]" (Kucharzik et al., 2021).

European Crohn's and Colitis Organisation and the European Society of Gastrointestinal and Abdominal Radiology (ECCO-ESGAR)

Working with the European Society of Gastrointestinal and Abdominal Radiology (ESGAR), ECCO has developed a list of laboratory parameters for the initial diagnosis of known IBD and the detection of its complications. These relevant provisions of these new diagnostic consensus guidelines are included below.

- "Statement 1.1. ECCO-ESGAR Diagnostics GL [2018]

  A single reference standard for the diagnosis of Crohn's disease [CD] or ulcerative colitis [UC] does not exist. The diagnosis of CD or UC is based on a combination of clinical, biochemical, stool, endoscopic, cross-sectional imaging, and histological investigations [EL5]"

- "Statement 1.2. ECCO-ESGAR Diagnostics GL [2018]

  Genetic or serological testing is currently not recommended for routine diagnosis of CD or UC [EL3]"

- "Statement 1.3. ECCO-ESGAR Diagnostics GL [2018]

  On diagnosis, complementary investigations should focus on markers of disease activity [EL2], malnutrition, or malabsorption [EL5]. Immunisation status should be assessed. Consider screening for latent tuberculosis [EL5]" (Maaser et al., 2019)

It should also be noted that "Serological markers may be used to support a diagnosis, though the accuracy of the best available tests [pANCA and ASCAs] is rather limited and hence ineffective at differentiating colonic CD from UC. Similarly, the additional diagnostic value of antiglycan and antimicrobial antibodies, such as anti-OmpC and CBir1, is small" (Maaser et al., 2019).

European Crohn's and Colitis Organisation (ECCO) and European Society of Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN)

This joint guideline was published regarding "Management of Paediatric Ulcerative Colitis" Although there was no mention of serological markers, the guideline did make this comment on "very early-onset inflammatory bowel disease presenting as colitis," which is as follows:

- "Unusual disease evolution, history of recurrent infections, HLH [hemophagocytic lymphocytic histiocytosis], and non-response to multiple IBD medications may indicate an underlying genetic defect which should prompt genetic and/or immunological analyses at any age during childhood" (Turner et al., 2018).

World Gastroenterology Organisation (WGO)

Concerning the use of p-ANCA and ASCA to diagnose UC and CD, the WGO states, "These tests are unnecessary as screening tests, particularly if endoscopy or imaging is going to be pursued for more definitive diagnoses. p-ANCA may be positive in Crohn's colitis and hence may not be capable of distinguishing CD from UC in otherwise unclassified colitis. ASCA is more specific for CD. These tests may have added value when there may be subtly abnormal findings, but a definitive diagnosis of inflammatory bowel disease is lacking. They may also be helpful if considering more advanced endoscopic techniques such as capsule endoscopy or double-balloon endoscopy, such that a positive ASCA test may provide stronger reasons for evaluating the small bowel." Later, the WGO also notes, "There are several other antibody tests, mostly for microbial antigens, that increase the likelihood of CD either singly, in combination, or as a sum score of the ELISA results for a cluster of antibodies. These tests are costly and not widely available. The presence of these antibodies, including a positive ASCA, would increase the likelihood that an unclassified IBD-like case represents Crohn's disease" (Bernstein et al., 2016).

Working Group of the North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition and the Crohn's and Colitis Foundation of America (NASPGHAN)

A clinical report (Bousvaros et al., 2007) noted that:

"A positive ANCA does not differentiate between UC and Crohn colitis."

"Genetic testing cannot as yet reliably differentiate UC from CD of the colon."

The Working Group also observed that in the largest study of prospective markers for UC, most patients remained seronegative for both ASCA and ANCA.

North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition (NASPGHAN)

NASPGHAN published a guideline regarding the management of patients with "Very Early-Onset Inflammatory Bowel Disease (VEO-IBD)". This guideline defines this cohort as a patient of the pediatric IBD population presenting at under 6 years of age. The guideline makes the following remarks on evaluation of IBD in this population:

- "…genetic sequencing is often necessary to identify the specific monogenic forms of VEO-IBD, or to confirm a suspected defect."
- "Targeted panels should be performed first in cases of infantile onset IBD, when the phenotype is consistent with a known defect, history of consanguinity, and abnormal immunology studies."
- "Currently, WES is most often performed in the setting of a negative targeted panel, however, there are select cases in which WES may be indicated instead of a targeted panel, such as those patients who present with a phenotype that is not previously described."
- "At this time, WGS should be reserved for cases in which WES is negative, yet there remains a high suspicion of a monogenic defect given the young age of onset, disease severity, family history, and complex phenotype including associated autoimmunity."
- "In general, the gene defects that have been detected with the highest frequency in patients with VEO-IBD can prompt specific targeted therapies that include: defects that lead to CGD (NADPH complex defects), IL-10R and XIAP" (Kelsen et al., 2019).

National Institute for Health and Care Excellence (NICE)

NICE does not mention any serological or genetic biomarkers in its reviews of management of UC or CD (NICE, 2019a, 2019b).

British Society of Gastroenterology (BSG)

The BSG published guidelines on the "management of inflammatory bowel disease [IBD] in adults." In it, they made the following comments regarding use of biomarkers in IBD:

- "…more evidence is also needed of the role of faecal calprotectin or other biomarkers as non-invasive surrogates for mucosal healing."
- "Further studies are required to evaluate the use of drug levels and biomarkers to determine personalized dosing for patients."
- "If a response [to treatment] is unclear, then measurement of biomarkers, serum C-reactive protein and faecal calprotectin, or comparison of disease activity scores or PROMs with baseline values, may be helpful."
- "We suggest that genetic testing for monogenic disorders should be considered in adolescents and young adults who have had early onset (before 5 years of age) or particularly aggressive, refractory or unusual IBD presentations (GRADE: weak recommendation, very low-quality evidence" (Lamb et al., 2019).

In 2021, the BSG released guidelines on management of irritable bowel syndrome. The BSG suggests that "all patients presenting with symptoms of IBS for the first time in primary care should have a full blood count, C reactive protein or erythrocyte sedimentation rate, coeliac serology and, in patients <45 years of age with diarrhea, a faecal calprotectin to exclude inflammatory bowel disease. Local and national guidelines for colorectal and ovarian cancer screening should be followed, where indicated" (Vasant et al., 2021).

World Society of Emergency Surgery and the American Association for the Surgery of Trauma

WSES and AAST released joint guidelines on the management of inflammatory bowel disease in the emergency setting. When assessing an acute abdomen in patients with IBD, "laboratory tests including full blood count, electrolytes, liver enzymes, inflammatory biomarkers such as erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP), and serum albumin and pre-albumin (to assess nutritional status and degree of inflammation) are mandatory" (De Simone et al., 2021).

Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
81401	Molecular pathology procedure, Level 2 (e.g., 2-10 SNPs, 1 methylated variant, or 1 somatic variant [typically using nonsequencing target variant analysis], or detection of a dynamic mutation disorder/triplet repeat)
81479	Unlisted molecular pathology procedure
82397	Chemiluminescent assay
83516	Immunoassay for analyte other than infectious agent antibody or infectious agent antigen; qualitative or semiquantitative, multiple step method
83520	Immunoassay for analyte other than infectious agent antibody or infectious agent antigen; quantitative, not otherwise specified
86021	Antibody identification; leukocyte antibodies
86036	Antineutrophil cytoplasmic antibody (ANCA); screen, each antibody
86037	Antineutrophil cytoplasmic antibody (ANCA); titer, each antibody
86255	Fluorescent noninfectious agent antibody; screen, each antibody
86671	Antibody; fungus, not elsewhere specified
88346	Immunofluorescence, per specimen; initial single antibody stain procedure
88350	Immunofluorescence, per specimen; each additional single antibody stain procedure (List separately in addition to code for primary procedure)
0164U	Gastroenterology (irritable bowel syndrome [IBS]), immunoassay for anti-CdtB and anti-vinculin antibodies, utilizing plasma, algorithm for elevated or not elevated qualitative results Proprietary test: ibs-smart™ Lab/Manufacturer: Gemelli Biotech
0176U	Cytolethal distending toxin B (CdtB) and vinculin IgG antibodies by immunoassay (ie, ELISA) Proprietary test: IBSchek® Lab/Manufacturer: Commonwealth Diagnostics International, Inc
0203U	Autoimmune (inflammatory bowel disease), mRNA, gene expression profiling by quantitative RT-PCR, 17 genes (15 target and 2 reference genes), whole blood, reported as a continuous risk score and classification of inflammatory bowel disease aggressiveness

	Proprietary test: PredictSURE IBD™ Test Lab/Manufacturer: KSL Diagnostics

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

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Revision History

Revision Date	Summary of Changes
09/06/2023	Reviewed and Updated: Updated the background, guidelines and recommendations, and evidence-based scientific references. Literature review did not necessitate any modifications to coverage criteria. The following edits were made for clarity: CC1 and CC2 edited for clarity and consistency

Lyme Disease Testing

Policy Number: AHS – G2143 – Lyme Disease Testing	Prior Policy Name and Number, as applicable: AHS-G2143-Lyme Disease
Initial Presentation Date: 2/10/2017 Revision Date: 03/06/2024	

Policy Description

Lyme disease is a common multisystem inflammatory disease caused by spirochetes of the family *Borreliaceae* transmitted through the bite of an infected tick of the genus *Ixodes* (Barbour, 2022). Lyme disease affects the skin in its early localized stage, and spreads to the joints, nervous system, and other organ systems in its later disseminated stages (Hu, 2022).

Related Policies

Policy Number	Policy Title
AHS-G2158	Testing for Mosquito- or Tick-Related Infections

Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) For individuals with symptoms of Lyme disease and a history of travel to a region endemic for Lyme (with or without a history of a tick bite), serologic testing (2-tier testing strategy using a sensitive enzyme

immunoassay (EIA) or immunofluorescence assay, followed by a western immunoblot assay or FDA-cleared second EIA assay) **MEETS COVERAGE CRITERIA**.

2) For individuals with a history of travel to a region endemic for Lyme, serologic testing (2-tier testing strategy using a sensitive enzyme immunoassay (EIA) or immunofluorescence assay, followed by a western immunoblot assay or FDA-cleared second EIA assay) **MEETS COVERAGE CRITERIA** in **any** of the following situations:
   a) For individuals with acute myocarditis/pericarditis of unknown cause.
   b) For individuals with meningitis, encephalitis, or myelitis.
   c) For individuals with painful radiculoneuritis.
   d) For individuals with mononeuropathy multiplex including confluent mononeuropathy multiplex.
   e) For individuals with acute cranial neuropathy.

3) Serologic testing **DOES NOT MEET COVERAGE CRITERIA** in **any** of the following situations:
   a) For individuals with an erythema migrans (EM) rash (patients with skin rashes consistent with EM who reside in or who have recently traveled to an endemic area should be treated for Lyme disease).
   b) To screen asymptomatic patients living in endemic areas.
   c) For individuals with non-specific symptoms only (e.g., fatigue, myalgias/arthralgias).
   d) For individuals with amyotrophic lateral sclerosis.
   e) For individuals with relapsing-remitting multiple sclerosis.
   f) For individuals with Parkinson's disease.
   g) For individuals with dementia or cognitive decline, or new-onset seizures.
   h) For individuals with psychiatric illness.

4) Detection of *Borrelia burgdorferi* by nucleic acid identification techniques (direct **or** amplified probe) **DOES NOT MEET COVERAGE CRITERIA**.

5) For individuals who have previously tested positive for Lyme disease, repeat serologic testing **DOES NOT MEET COVERAGE CRITERIA**.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

6) All other testing for *Borrelia burgdorferi* not described above **DOES NOT MEET COVERAGE CRITERIA**.

7) For the diagnosis of Lyme disease, testing of the individual tick **DOES NOT MEET COVERAGE CRITERIA**.

Table of Terminology

Term	Definition
AAN	The American Academy of Neurology

AAP	American Academy of Pediatrics
ACR	The American College of Rheumatology
ACEIA	Antibody capture enzyme immunoassay
CCDR	Canada Communicable Disease Report
CD57	Cluster designation 57
CDC	Centers for Disease Control and Prevention
CLIA ’88	Clinical Laboratory Improvement Amendments of 1988
CMS	Centers for Medicare and Medicaid Services
CNS	Central nervous system
CPS	Canadian Paediatric Society
CSF	Cerebrospinal fluid
EIA	Enzyme immunoassay
ELISA	Enzyme-linked immunosorbent assay
EM	Erythema migrans
FDA	Food and Drug Administration
HDPCR	High-definition polymerase chain reaction
IDEG	Infectious Disease Expert Group
IDSA	The Infectious Diseases Society of America
IFA	Immunofluorescence assay
IgG	Immunoglobulin G
IgM	Immunoglobulin M
LD	Lyme disease
LDT	Laboratory developed test
LNB	Lyme neuroborreliosis
MTTT	Modified two-tiered testing
NICE	National Institute for Health and Care Excellence
PBMC	Peripheral blood mononuclear cell
PCR	Polymerase chain reaction
PHAC	Public Health Agency of Canada
PNS	Peripheral nervous system
PPV	Positive predictive value
PTLDS	Post-Lyme disease syndrome
RUO	Research use only
STTT	Standardized two-tier testing
TBP	Tick-borne pathogen
WB-RTPCR	Whole blood real-time polymerase chain reaction
xVFA	Multiplexed vertical flow assay

Scientific Background

Lyme disease can be caused by several species in the spirochete family *Borreliaceae;* however, infection in North America is predominately caused by *B. burgdorferi*. Much less commonly, in the upper midwestern United States, cases have been associated with *B. mayonii* (Mead & Schwartz, 2022; Pritt et al., 2016). The taxonomic classification system for this species is undergoing revision, and the genus name may be represented as either *Borrelia* or *Borreliella* (Adeolu & Gupta, 2014; Margos et al., 2017). *Borrelia burgdorferi* occurs naturally in reservoir hosts, including small mammals and birds (Hyde, 2017). *Ixodes scapularis* and *I. pacificus* become infected with *B. burgdorferi* while feeding on the blood of natural reservoir hosts. Transmission to humans results from the bite of an infected tick (Bacon et al., 2008). Spirochete transmission times and virulence depend upon the tick and *Borrelia* species, and infection can never be excluded after a tick bite irrespective of the estimated duration of attachment time (Cook, 2015).

In the earliest stage of Lyme disease, *B. burgdorferi* disseminates from the site of the tick bite resulting in the colonization of dermal tissue and localized infection characterized by a painless bulls-eye rash called erythema migrans, experienced by approximately 70–80% of patients at the site of the tick bite. This is accompanied by non-specific flu-like symptoms, including headache, neck stiffness, malaise, fatigue, myalgia, and fever. During localized infection, the number of *B. burgdorferi* cells increases in the dermal tissue. If left untreated, *B. burgdorferi* can disseminate from the site of the tick bite through the bloodstream and/or lymphatic system to invade and colonize various tissues days to weeks after infection. This can affect the heart, joints, and nervous system. Months to years after exposure to *B. burgdorferi,* affected individuals can experience different manifestations, including neuroborreliosis, Lyme carditis, and arthritis (Hyde, 2017).

The CDC reports that about 476,000 Americans are diagnosed with Lyme disease each year, but they estimate that only about 300,000 people get Lyme disease each year. The CDC notes that these numbers likely differ because the 476,000 people treated for Lyme disease, and patients are often treated presumptively and without proper testing (CDC, 2021b).

Even following antibiotic treatment, a subset of patients continue to present with arthritic symptoms; this has been designated as postinfectious, antibiotic-refractory Lyme arthritis (Hyde, 2017). The term "post-Lyme disease syndrome" (PTLDS) is often used to describe the nonspecific symptoms (such as headache, fatigue, and arthralgias) that may persist for months after treatment of Lyme disease. For the majority of patients, these symptoms improve gradually over six months to one year (Hu, 2022). Weitzner et al. (2015) found that "PTLDS may persist for over 10 years in some patients with culture-confirmed early Lyme disease. Such long-standing symptoms were not associated with functional impairment or a particular strain of *B. burgdorferi*."

The diagnosis of Lyme disease is based on an individual's history of possible exposure to ticks, the presence of characteristic signs and symptoms, and blood test results (Hu, 2022). Direct detection of *Borrelia burgdorferi* has limited applications (Marques, 2015). Thus, most laboratory confirmation of Lyme disease involves the detection of antibody responses against *B. burgdorferi* in serum (Schriefer, 2015). Serology testing is not recommended for patients who do not have symptoms typical of Lyme disease (Marques, 2015), as current assays do not distinguish between active and past infection, thus a positive result is more likely to be a false positive. Early diagnosis of erythema migrants should be made without testing because the lesion appears prior to development of a diagnostic, adaptive immune response (Hu, 2022).

Serological testing using the two-tier algorithm, comprising a first screening enzymatic immunoassay (EIA), followed by a confirmatory Western blot test, is the gold standard for Lyme disease diagnoses (Bunikis & Barbour, 2002; Hu, 2022; John & Taege, 2019). Standardized two-tier testing (STTT) is the recommended diagnostic technique for Lyme disease in clinical practice (CDC, 2021a). Although STTT detection of early localized infection is poor, STTT detection of late disease is excellent (Schriefer, 2015). Evidence of seronegative late Lyme disease is unconvincing (Halperin, 2015). A systematic review has shown that the sensitivity of serology for Lyme disease in early localized infection is 50%, but the algorithm performs well in late stages of the infection, where the sensitivity approaches 100% (Waddell et al., 2016).

On July 29, 2019, the FDA approved several Lyme disease serologic assays, including ZEUS ELISA, allowing for an EIA rather than Western blot as the second test in the two tier algorithm (CDC, 2019). ZEUS ELISA is a Modified Two-Tiered Testing (MTTT) Algorithm that replaces the second-tier Western blot with a more sensitive and specific methodology, such as ELISA. According to ZEUS Scientific, MTTT reduces the number of missed clinically positive patient samples and improves lab efficiency (ZEUS Scientific, 2019). Compared to the traditional STTT, the MTTT algorithms improve sensitivity to detect early infections and have equivalent sensitivity for detecting late-stage infections and comparable specificity. In addition, MTTT may have the benefit of improved sensitivity in identifying positive cases in patients infected with related strains of *Borrelia*. In a study by Davis, one case of infection with a European genospecies of *Borrelia* was detected by MTTT, which was missed by STTT (Davis et al., 2020). The Canada Communicable Disease Report (CCDR) agrees with the FDA recommendation, advising that "Diagnostic improvements in sensitivity of [Lyme disease] testing without significant loss of specificity have been consistently reported when MTTT is compared with STTT in studies conducted in highly [Lyme disease] endemic regions" (CCDR, 2020).

Polymerase chain reaction (PCR) testing may be useful in the early stages of a Lyme disease infection before an immune response occurs and is also helpful when testing for reinfection. Other potential techniques for Lyme disease diagnostics include cell culture, ELISA, urine testing, and multiplex testing techniques (John & Taege, 2019).

Proprietary Testing

Other diagnostic tests have been created but not widely validated (Hu, 2022). For instance, Wormser et al. (2013) evaluated a C6 enzyme-linked immunosorbent assay (ELISA) as a single-step, serodiagnostic test that uses a reference standard of two-tier testing. This test provided increased sensitivity in early Lyme disease with comparable sensitivity in later manifestations of the disease. Four hundred and three samples were compared to the sensitivities of the traditional two-tier tests, and the C6 ELISA was measured to have a 66.5% sensitivity and a 35.2% sensitivity, both of which were more sensitive than the individual steps of the STTT approach. The specificity was evaluated with over 2200 blood donors, and the C6 ELISA was evaluated at 98.9% specificity (Wormser et al., 2013).

Urine testing for diagnosis of Lyme disease is available from multiple laboratories. For example, Igenex (2017b) claims that the urine tests "are useful during the acute phase of infection before antibodies are present, in seronegative patients, in patients with vague symptoms of long duration, and previously-treated patients with recurring symptoms." However, the American Academy of Pediatrics (AAP) asserts that "A number of tests for Lyme disease have been found to be invalid on the basis of independent testing or to be too nonspecific to exclude false-positive results", including "urine tests for B burgdorferi, CD57 assay, novel culture techniques, and antibody panels that differ from those recommended as part

of standardized 2-tier testing" (AAP, 2021). The CDC also includes urine testing for Lyme disease within their list of laboratory tests that are not recommended (CDC, 2023).

Igenex's proprietary Immunoblot has been used to detect IgM and IgG antibodies to diagnose Lyme disease. From the sample report, Igenex has stated that "Recombinant *B. burgdorferi* species antigens are sprayed at specific positions onto a nitrocellulose membrane and cut into strips. These strips are used to detect *B. burgdorferi* specific antibodies in patient serum" (Igenex, 2017b). Eight total species of *Borrelia* are detected by this test; based on 174 samples, the ImmunoBlot was found to have a sensitivity of 90.9% and specificities of 98% (IgM) and 98.7% (IgG) (Igenex, 2017b). Igenex also has a PCR-based test for the detection of *B. burgdorferi*. Four hundred and two positive samples for *B. burgdorferi* were evaluated based on Igenex's proprietary PCR test and the CDC diagnostic criteria (the traditional two-tiered test). Out of the 402 samples, 236 were considered positive by the proprietary PCR test and 70 were considered positive per the CDC criteria (Igenex, 2017a).

Clinical Utility and Validity

Waddell et al. (2016) assessed the accuracy of the traditional diagnostic tests of Lyme disease. A total of 11 studies with 34 lines of data were evaluated for the overall accuracy. The overall sensitivity was found to be 82%, and the overall specificity was found to be 94.2%. Fifteen studies were examined for Stage 1 of Lyme disease, and the sensitivity was found to be 54%; however, the specificity was calculated to be 96.8%. Stage 2 (five studies, six lines) had a sensitivity of 79.1% and specificity of 97.7%, and Stage 3 (nine studies, 20 lines) had a sensitivity of 94.7% and specificity of 96.1%. The CDC immunoblots (second tier, two studies, four lines) were estimated at 91% sensitivity and 99% specificity (Waddell et al., 2016).

Joung et al. (2019) note that while the CDC recommends serological methods for Lyme disease testing, it is expensive (over $400/test) and can take longer than 24 hours to obtain results; therefore, a cost-effective and rapid assay was developed to address these challenges. This assay can detect early stage Lyme disease and "assays for antibodies specific to seven *Borrelia* antigens and a synthetic peptide in a paper-based multiplexed vertical flow assay (xVFA)"; the specificity of this test was identified at 87% and sensitivity at 90.5% (Joung et al., 2019).

Shakir et al. (2019) used a total of 379 whole blood samples to evaluate ChromaCode's Research Use Only (RUO) nine target High-Definition PCR (HDPCR™) Tick-Borne Pathogen (TBP) panel. Results were compared to clinically validated real-time PCR assays and laboratory developed tests. The final positive percent agreement and negative percent agreement "for the TBP panel was 97.7% (95% CI 95.2% - 99.0%) and 99.6% (95% CI 99.3% - 99.8%), respectively, with an overall agreement of 99.5% (95% CI 99.2% -99.7%)" with the laboratory developed tests" (Shakir et al., 2019).

Nigrovic et al. (2019) evaluated the Lyme disease PCR test compared to the traditional two-tier assessment method (a positive or equivocal EIA and a positive immunoblot test). In total, 124 were tested and 54 had Lyme disease. However, only 23 of the Lyme disease patients had a positive PCR test, giving a sensitivity of 41.8% and specificity of 100% (Nigrovic et al., 2019). These results show that the Lyme disease PCR test has low sensitivity.

Davis et al. (2020) evaluated the effectiveness of the MTTT algorithm compared to the STTT algorithm. Modified two-tiered testing (MTTT) algorithm uses a second enzyme immunoassay (EIA) instead of the immunoblots for samples that test positive or equivocal on the first EIA. Retrospective chart reviews were performed on 10,253 specimens tested for Lyme disease (LD) serology. "Patients were classified as having Lyme disease if they had a positive STTT result, a negative STTT result but symptoms consistent

with Lyme disease, or evidence of seroconversion on paired specimens" (Davis et al., 2020). Of the 10,253 specimens, 9,806 (95.6%) were negative for Lyme disease and 447 patients tested positive. Of the 447 patients, 227 were classified as patients with Lyme disease. "Of the 227 patients classified as having LD, 65 (28.6%) had early localized infections, 67 (29.5%) had early disseminated infections, 26 (11.5%) had late LD, 61 (26.9%) had evidence of old infections, and 8 (3.5%) had posttreatment LD syndrome. Of the remaining 63 patients with early localized disease, 16 (25.4%) were positive by MTTT but negative by STTT. The MTTT identified an additional four (6.6%) cases of early disseminated infection and one case (3.8%) in late LD" (Davis et al., 2020). Overall, MTTT identified additional cases in early localized and early disseminated infections and detected 25% more early infections with a specificity of 99.56% (99.41 to 99.68%) compared to the STTT (Davis et al., 2020).

van Gorkom et al. (2020) evaluated the utility of an in-house and a commercial enzyme-linked immunosorbent spot (ELISpot) assay for the diagnosis of Lyme neuroborreliosis (LNB). Peripheral blood mononuclear cells (PBMCs) were isolated from eighty-seven patients diagnosed with LNB at Diakonessenhuis Hospital, Utrecht, and the St Antonius Hospital, Nieuwegein, the Netherlands between March 2014 and November 2017. In-house *Borrelia* ELISpot assay and the commercial LymeSpot assay. However, it was found that both tests performed unsatisfactorily—the sensitivity for the *Borrelia* ELISpot yielded a sensitivity of 61.1% (95% CI: 38.9-77.8%) and a specificity of 66.7(42.0-81.2%), while the LymeSpot assay produced 66.7% (95% CI: 44.4-88.9%) and 59.4% (95% 44.9-72.5%), respectively. Moreover, low PPVs for ELISpot and LymeSpot were observed (30.6% vs. 29.7%, respectively), further corroborate their poor diagnostic performance. The researchers do acknowledge a few shortcomings in their study, namely that the isolation procedure for the PBMC deviated from that of the LymeSpot assay—however, the deviations from protocol were allowed for the technician to minimize differences when comparing across assays to allow for fairer comparison of results. Though this was the case, they believe still that the deviations "from the recommended protocol are not critical", and as such they uphold "the conclusion stands that both ELISpot assays cannot help to diagnose active LNB" (van Gorkom et al., 2020).

Sabin et al. (2023) compared the MTTT algorithm to the STTT. The authors compared samples from 320 patients. "The MTTT confirmed the illness in 116 subjects (36%, P = 0.007), and 30 (26%) were negative by the STTT." MTTT sensitivity was increased in early infection, but insufficiently sensitive to non-*Borrelia* species infections. The authors concluded that "Routine adoption of MTTT would improve sensitivity for early Lyme disease attributable to B. burgdorferi, but may not capture illness attributed to B. mayonii and B. miyamotoi" (Sabin et al., 2023).

Pratt et al. (2022) believed that the concurrent use of molecular and serologic testing could broaden the diagnostic window for early Lyme disease. Of the 33199 specimens submitted for review by antibody capture EIA and WB-RTPCR, 1379 tested positive, and of those positive, "1,179 were positive by serology only, 131 were positive by molecular testing only, and 69 were positive by both serology and molecular testing." Overall, they found that "4.2% of all specimens were positive and nearly 10% were detected by WB-RTPCR alone." The authors reported that "Of the 131 specimens that tested positive for *B burgdorferi* DNA only, 29 had follow-up samples submitted for follow-up serology testing". Most importantly, "Eighty-six percent (25/29) of the patients with follow-up testing demonstrated seroconversion, 3% (1/29) were equivocal, and 10% (3/29) tested negative" (Pratt et al., 2022). The researchers also examined "2526 specimens submitted for concurrent MTTT and molecular testing" and found that "The two data sets showed a similar percentage of molecular-positive, serology-negative results (8.7% for MTTT and 9.5% for ACEIA)". Moreover, using the χ^2 test, they found "no statistically significant difference between the antibody-capture and MTTT data sets was observed when analyzing the Lyme-positive results" ($\chi^2 = 0.2765$, $P = .871$). Consequently, it was concluded that "WB-RTPCR, in

clinically suspected cases of ELD, can identify *B burgdorferi* infection that serology testing could otherwise miss". Though a retrospective review of paired samples was used to confirm their results, the lack of clinical information to associate with the results motivates the need for a future prospective study (Pratt et al., 2022).

Guidelines and Recommendations

Centers for Disease Control and Prevention (CDC)

The CDC currently recommends a two-step process when testing blood for evidence of antibodies against the Lyme disease bacteria. Both steps can be done using the same blood sample.

- **The first step** uses a testing procedure called "EIA" (enzyme immunoassay) or rarely, an "IFA" (indirect immunofluorescence assay).
- **If this first step is negative**, no further testing of the specimen is recommended.
- **If the first step is positive** or indeterminate (sometimes called "equivocal"), the second step should be performed.
- **The second step** uses a test called an immunoblot test, commonly, a "Western blot" test.
- Results are considered positive only if the EIA/IFA and the immunoblot are both positive (CDC, 2021a; Mead et al., 2019).

CDC Guidelines on Non-Recommended Lab Tests:

Some laboratories offer Lyme disease testing using assays whose accuracy and clinical usefulness have not been adequately established. Examples of unvalidated tests include

- Capture assays for antigens in urine
- Immunofluorescence staining, or cell sorting of cell wall-deficient or cystic forms of *B. burgdorferi*
- Lymphocyte transformation tests
- Quantitative CD57 lymphocyte assays
- "Reverse Western blots"
- IgM or IgG tests without a previous enzyme immunoassay

The CDC additionally notes that

- If a laboratory uses "in-house" criteria for interpretation of FDA-cleared tests for Lyme disease, this indicates the laboratory has modified the test and the clinical validity and safety is not certain.
- Test results for Lyme disease should always be interpreted in the broader context of a person's illness and medical history, exposure likelihood, and other test results.
- Do not seek testing without consulting a healthcare provider (CDC, 2023).

In the 2019 update concerning the CDC recommendations for serologic diagnosis of Lyme disease, they state, "When cleared by FDA for this purpose, serologic assays that utilize EIA rather than western immunoblot assay in a two-test format are acceptable alternatives for the laboratory diagnosis of Lyme disease. Based on the criteria established at the 1994 Second National Conference on Serologic Diagnosis of Lyme Disease, clinicians and laboratories should consider serologic tests cleared by FDA as CDC-recommended procedures for Lyme disease serodiagnosis" (Mead et al., 2019).

The Infectious Diseases Society of America (IDSA), The American Academy of Neurology (AAN), and The American College of Rheumatology (ACR)

The IDSA, AAN and ACR have published clinical practice guidelines for the prevention, diagnosis, and treatment of Lyme disease. The guidelines include the following statements:

- Following a tick bite, "We recommend submitting the removed tick for species identification. (good practice statement)
- We recommend against testing a removed *Ixodes* tick for *B. burgdorferi* (strong recommendation, moderate quality evidence). The presence or absence of *B. burgdorferi* in an Ixodes tick removed from a person does not reliably predict the likelihood of clinical infection.
- We recommend against testing asymptomatic patients for exposure to *B. burgdorferi* following an *Ixodes* spp. tick bite (strong recommendation, moderate-quality evidence).
- In patients with potential tick exposure in a Lyme disease endemic area who have 1 or more skin lesions compatible with erythema migrans, we recommend clinical diagnosis rather than laboratory testing (strong recommendation, moderate quality evidence).
- In patients with 1 or more skin lesions suggestive of, but atypical for erythema migrans, we suggest antibody testing performed on an acute-phase serum sample (followed by a convalescent-phase serum sample if the initial result is negative) rather than currently available direct detection methods such as polymerase chain reaction (PCR) or culture performed on blood or skin samples (weak recommendation, low-quality evidence). Comment: If needed, the convalescent-phase serum sample should be collected at least 2–3 weeks after collection of the acute-phase serum sample.
- When assessing patients for possible Lyme neuroborreliosis involving either the peripheral nervous system (PNS) or central nervous system (CNS), we recommend serum antibody testing rather than PCR or culture of either cerebrospinal fluid (CSF) or serum (strong recommendation, moderate-quality evidence).
- If CSF testing is performed in patients with suspected Lyme neuroborreliosis involving the CNS, we (a) recommend obtaining simultaneous samples of CSF and serum for determination of the CSF: serum antibody index, carried out by a laboratory using validated methodology, (b) recommend against CSF serology without measurement of the CSF: serum antibody index, and (c) recommend against routine PCR or culture of CSF or serum (strong recommendation, moderate-quality evidence).
- In patients presenting with 1 or more of the following acute disorders: meningitis, painful radiculoneuritis, mononeuropathy multiplex including confluent mononeuropathy multiplex, acute cranial neuropathies (particularly VII, VIII, less commonly III, V, VI, and others), or in patients with evidence of spinal cord (or rarely brain) inflammation, the former particularly in association with painful radiculitis involving related spinal cord segments, and with epidemiologically plausible exposure to ticks infected with *B. burgdorferi*, we recommend testing for Lyme disease (strong recommendation, moderate-quality evidence).
- In patients with typical amyotrophic lateral sclerosis, relapsing-remitting multiple sclerosis, Parkinson's disease, dementia or cognitive decline, or new-onset seizures, we recommend against routine testing for Lyme disease (strong recommendation, low-quality evidence).
- In patients with neurological syndromes other than those listed… in the absence of a history of other clinical or epidemiologic support for the diagnosis of Lyme disease, we recommend against screening for Lyme disease (strong recommendation, low-quality evidence)
- In patients presenting with nonspecific magnetic resonance imaging white matter abnormalities confined to the brain in the absence of a history of other clinical or epidemiologic support for the diagnosis of Lyme disease, we suggest against testing for Lyme disease (weak recommendation, low-quality evidence).

- In patients with psychiatric illness, we recommend against routine testing for Lyme disease (strong recommendation, low-quality evidence).
- In children presenting with developmental, behavioral, or psychiatric disorders, we suggest against routinely testing for Lyme disease (weak recommendation, low-quality evidence).
- In patients with acute myocarditis/pericarditis of unknown cause in an appropriate epidemiologic setting, we recommend testing for Lyme disease (strong recommendation, low-quality evidence)
- In patients with chronic cardiomyopathy of unknown cause, we suggest against routine testing for Lyme disease (weak recommendation, low-quality evidence)
- When assessing for possible Lyme arthritis, we recommend serum antibody testing over PCR or culture of blood or synovial fluid/tissue (strong recommendation, moderate quality of evidence)
- In seropositive patients for whom the diagnosis of Lyme arthritis is being considered but treatment decisions require more definitive information, we recommend PCR applied to synovial fluid or tissue rather than *Borrelia* culture of those samples (strong recommendation, moderate quality of evidence)".

The guideline also made several relevant comments on the above recommendations:

- The guideline commented that knowing tick characteristics (such as "species, life stage, and an assessment of the degree of blood engorgement") is helpful for early guidance, such as antibiotic management.
- "Serologic testing of asymptomatic patients following a tick bite does not help with treatment decisions."
- "Association of Lyme disease with meningitis, cranial neuritis, radiculoneuritis, and other forms of mononeuropathy multiplex is well established…The few systematic studies that have been performed have failed to identify consistent associations between Lyme disease and amyotrophic lateral sclerosis, multiple sclerosis, Alzheimer's disease, or Parkinson's disease…These recommendations place a high value on avoiding false positive Lyme disease test results, which can delay appropriate medical evaluation and treatment of other disorders and lead to unnecessary antibiotic exposure and potential side effects."
- "The main disadvantage of this approach [the traditional 'two-tiered approach' is that seroreactivity after successfully treated Lyme borreliosis may persist for years, complicating test interpretation in patients with known previous exposure and/or in patients from highly endemic areas where background seroprevalence is substantial. In such patients, after seroreactivity has been demonstrated, synovial fluid or synovial tissue B. burgdorferi PCR may improve diagnostic specificity" (Lantos et al., 2021).

The American College of Rheumatology (ACR)

The ACR also recommends that "the musculoskeletal manifestations of Lyme disease include brief attacks of arthralgia or intermittent or persistent episodes of arthritis in one or a few large joints at a time, especially the knee. Lyme testing in the absence of these features increases the likelihood of false positive results and may lead to unnecessary follow-up and therapy. Diffuse arthralgias, myalgias or fibromyalgia alone are not criteria for musculoskeletal Lyme disease" (ACR, 2013).

Committee on Infectious Diseases of the American Academy of Pediatrics, 32nd/ Edition

The Committee on Infectious Diseases of the American Academy of Pediatrics states that "Diagnosis of Lyme disease rests first and foremost on the recognition of a consistent clinical illness in people who have had plausible geographic exposure. Early Lyme disease in patients with erythema migrans is

diagnosed clinically on the basis of the characteristic appearance of this skin lesion. Although erythema migrans is not pathognomonic for Lyme disease, it is highly distinctive and characteristic. In areas with endemic Lyme disease, it is expected that the vast majority of erythema migrans occurring in the appropriate season is attributable to *B burgdorferi* infection" (AAP, 2021).

The AAP report a 2-tier serologic algorithm as the standard testing method for Lyme disease, in which "The initial screening test identifies antibodies to a whole-cell sonicate, to peptide antigen, or to recombinant antigens of B burgdorferi using an enzyme-linked immunosorbent assay (ELISA or EIA) or immunofluorescent antibody (IFA) test. It should be noted that clinical laboratories vary somewhat in their description of this test. It may be described as "Lyme ELISA," "Lyme antibody screen," "total Lyme antibody," or "Lyme IgG/IgM." Many commercial laboratories offer EIA/IFA with reflex to Western immunoblot if the first-tier assay result is positive or equivocal. Although the initial EIA or IFA test result may be reported quantitatively, its sole importance is to categorize the result as negative, equivocal, or positive"(AAP, 2021).

Then, "If the first-tier EIA result is negative, the patient is considered seronegative and no further testing is indicated. If the result is equivocal or positive, then a second-tier test is required to confirm the result. There are 2 options for second tier testing: (1) a western immunoblot, which is the standard 2-tiered testing algorithm; or (2) an EIA test that has been specifically cleared by FDA for use as a second-tier confirmatory test, which is the modified 2-tiered testing algorithm". However, the AAP also reports that "Some assays marketed in the United States have reduced sensitivity for European strains of B burgdorferi. For patients potentially infected in Europe, check with the test provider or laboratory director to select tests that have been validated for this purpose" (AAP, 2021).

The AAP Red Book also delineates for whom and when testing is appropriate.

They caution against the use of serologic testing for Lyme disease in children "without symptoms or signs suggestive of Lyme disease and plausible geographic exposure."

They recommend against Western immunoblot testing "the initial EIA or IFA test result is negative or without a prior EIA or IFA test, because specificity of immunoblot diminishes if the test is performed alone."

"No polymerase chain reaction (PCR) test for *B burgdorferi* currently is cleared by the FDA. PCR testing of joint fluid from a patient with Lyme arthritis often yields positive results and can be informative in establishing a diagnosis of Lyme arthritis. The role of a PCR assay on blood is not well established; test results usually are negative in early and late Lyme disease and is not recommended routinely. Yield of PCR testing on cerebrospinal fluid samples from patients with neuroborreliosis is too low to be useful in excluding this diagnosis."

"A number of tests for Lyme disease have been found to be invalid on the basis of independent testing or to be too nonspecific to exclude false-positive results. These include urine tests for *B burgdorferi*, CD57 assay, novel culture techniques, and antibody panels that differ from those recommended as part of standardized 2-tier testing. Although these tests are commercially available from some clinical laboratories, they are not FDA cleared and are not appropriate diagnostic tests for Lyme disease" (AAP, 2021).

Moreover, the interpretation of the results of diagnostic testing can be fraught with difficulties. The notable scenarios are reported below.

"Some patients treated with antimicrobial agents for early Lyme disease never develop detectable antibodies against B burgdorferi; they are cured and are not at risk of late disease. Development of antibodies in patients treated for early Lyme disease does not indicate lack of cure or presence of persistent infection. Ongoing infection without development of antibodies ("seronegative Lyme") has not been demonstrated. Most patients with early disseminated disease and virtually all patients with late disease have antibodies against B burgdorferi. Once such antibodies develop, they may persist for many years. Tests for antibodies should not be repeated or used to assess success of treatment."

"A positive IgM immunoblot result can be falsely positive. The IgM assay is useful only for patients in the first 4 weeks after symptom onset. The IgM immunoblot result should be disregarded (or, if possible, not ordered) in patients who have had symptoms for longer than 4 weeks, or symptoms consistent with late Lyme disease, because false-positive IgM assay results are common, and because most untreated patients with disseminated Lyme disease will have a positive IgG result by week 4 of symptoms."

"Lyme disease test results for B burgdorferi in patients treated for syphilis or other spirochete diseases are difficult to interpret."

"Standardized 2-tier testing can be expected to have positive results in patients with *B mayonii* infection", as "patients with *B mayonii* infection develop a serologic response similar to that of patients infected with B burgdorferi" (AAP, 2021).

National Institute for Health and Care Excellence (NICE)

NICE recommends diagnosis without laboratory testing in patients with erythema migrans. For patients without erythema migrans, NICE states to consider using an ELISA test. If this ELISA is positive or equivocal, then an immunoblot may be performed. If both tests are positive, then Lyme disease may be diagnosed (NICE, 2018).

NICE also published guidelines in 2019 with the following recommendations:

- "People presenting with erythema migrans are diagnosed and treated for Lyme disease based on clinical assessment, without laboratory testing.
- People with suspected Lyme disease without erythema migrans who have a negative enzyme-linked immunosorbent assay (ELISA) test carried out within 4 weeks of their symptoms starting may have the test repeated 4 to 6 weeks later if Lyme disease is still suspected" (NICE, 2019).

NICE also produced a diagnostic algorithm with the following recommendations:

- "If Lyme disease is still suspected in people with a negative ELISA who have had symptoms for 12 weeks or more, perform an immunoblot test.
- Carry out an immunoblot test, despite an initial negative ELISA, when there is clinical suspicion of Lyme disease. Diagnose Lyme disease in people with symptoms of Lyme disease and a positive immunoblot test.
- If the immunoblot test for Lyme disease is negative (regardless of the ELISA result) but symptoms persist, consider a discussion with or referral to a specialist, to: review whether further tests may be needed for suspected Lyme disease, for example, synovial fluid aspirate or biopsy, or lumbar puncture for cerebrospinal fluid analysis or consider alternative diagnoses (both infectious, including other tick-borne diseases, and non-infectious).

- Initial testing with a combination IgM and IgG ELISA for Lyme disease should be offered because the evidence generally showed better accuracy (both sensitivity and specificity) for combined tests compared to IgM-only and IgG-only tests. The evidence was best for tests based on purified or recombinant antigens derived from the VlsE protein or its IR6 domain peptide (such as a C6)."

This diagnostic algorithm was primarily based off of NICE's 2018 guidelines (NICE, 2018).

Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). As an LDT, the U. S. Food and Drug Administration has not approved or cleared this test; however, FDA clearance or approval is not currently required for clinical use.

Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
86617	Antibody; Borrelia burgdorferi (Lyme disease) confirmatory test (eg, Western Blot or immunoblot)
86618	Antibody; Borrelia burgdorferi (Lyme disease)
87475	Infectious agent detection by nucleic acid (DNA or RNA); Borrelia burgdorferi, direct probe technique
87476	Infectious agent detection by nucleic acid (DNA or RNA); Borrelia burgdorferi, amplified probe technique
0041U	Borrelia burgdorferi, antibody detection of 5 recombinant protein groups, by immunoblot, IgM Proprietary test: Lyme ImmunoBlot IgM Lab/Manufacturer: IGeneX Inc
0042U	Borrelia burgdorferi, antibody detection of 12 recombinant protein groups, by immunoblot, IgG Proprietary test: Lyme ImmunoBlots IgG Lab/Manufacturer: IGeneX Inc
0316U	Borrelia burgdorferi (Lyme disease), OspA protein evaluation, urine Proprietary test: Lyme Borrelia Nanotrap® Urine Antigen Test Lab/Manufacturer: Galaxy Diagnostics Inc

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

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Revision History

Revision Date	Summary of Changes
03/06/2024	Reviewed and Updated: Updated the background, guidelines and recommendations, and evidence-based scientific references. Literature review did not necessitate any modifications to coverage criteria.
03/01/2023	Reviewed and Updated: Updated the background, guidelines and recommendations, and evidence-based scientific references. Literature review did not necessitate any modifications to coverage criteria. The following edits were made for clarity: Title changed to "Lyme Disease Testing" All CC edited for clarity and consistency. Due to redundancy with CC4: " 4) Detection of Borrelia burgdorferi by nucleic acid identification techniques (direct or amplified probe) DOES NOT MEET COVERAGE CRITERIA.", removed CC6: "1) Repeat PCR-based direct detection of Borrelia burgdorferi DOES NOT MEET COVERAGE CRITERIA in the following situations: a) As a justification for continuation of IV antibiotics beyond one month in patients with persistent symptoms. b) As a technique to follow a therapeutic response. c) Via urine sample."
03/09/2022	Annual review: Updated background, guidelines and recommendations, and evidence-based scientific references. Literature review did not necessitate modification to coverage criteria. CPT Changes –Added 0316U.
03/03/2021	Annual Review: Updated background, guidelines, and evidence-based scientific references. Literature review necessitated the following changes to the CC: Per 2020 IDSA/AAN/ACR, CC2 was reworded for clarity and following acute disorders were added: meningitis/encephalitis/myelitis, painful radiculoneuritis, mononeuropathy multiplex including confluent mononeuropathy multiplex, acute cranial neuropathy. Per 2020 IDSA/AAN/ACR addition of "in patients with amyotrophic lateral sclerosis, in patients with relapsing-remitting multiple sclerosis, in patients with Parkinson's disease, in patients with dementia or cognitive decline, or new-onset seizures, in patients with psychiatric illness" to CC3.
03/10/2020	Annual Review: Updated background, guidelines, and evidence-based scientific references. Added CC stating that serologic testing (2-tier testing strategy) for individuals with acute myocarditis/pericarditis of unknown cause **MCC** based on 2019 IDSA/AAN/ACR guidelines. Added statement regarding to the lack of published references for the old E&I CCs and changed the old E&I CCs to DNMCC. Per CAB decision, added CC stating, "Repeat serologic testing DOES NOT MEET COVERAGE CRITERIA in individuals who have tested positive previously since positive results may not distinguish between past and possible current infection(s)."
03/01/2019	Annual Review: Updated background, guidelines, and evidence-based scientific references. Added CCs stating that urine assays, including urinary-based antigen capture assays, and panel immunoblot testing are **E&I.** Modified CC by removing specific

	instances where PCR testing is not allowed. CC now states that PCR-based detection of *B. burgdorferi* **DNMCC**. Added 0041U – 0044U.
03/16/2018	Annual Review: Background and Evidence-based Scientific References were updated. Literature review did not necessitate any modification to coverage criteria. No coding changes.
02/10/2017	Initial presentation.

Metabolite Markers of Thiopurines Testing

Policy Number: AHS – G2115 – Metabolite Markers of Thiopurines Testing	Prior Policy Name and Number, as applicable: • AHS-G2115 Pharmacogenomic and Metabolite Markers for Thiopurines
Initial Presentation Date: 09/18/2015 Revision Date: February 1, 2025	

Policy Description

Thiopurines are a class of purine antimetabolite immunomodulators with diverse clinical applications in treatment of autoimmune disorders, transplant rejection, and acute lymphoblastic leukemia (Belmont, 2022). Their therapeutic efficacy, bone marrow toxicity, and liver toxicity have been reported to be related to levels of their downstream metabolites. Due to their complex metabolism, patient response varies considerably between individuals, both in achieving therapeutic drug levels as well as in developing adverse reactions (Bradford & Shih, 2011).

Please note that this policy discusses the monitoring of thiopurine metabolite levels in individuals. For guidance on pharmacogenetic testing prior to therapy, please refer to AHS-M2021 Pharmacogenetic Testing.

Related Policies

Policy Number	Policy Title
AHS-M2021	Pharmacogenetic Testing

Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) One-time phenotypic analysis of the enzyme thiopurine methyltransferase (TPMT) **MEETS COVERAGE CRITERIA** for **any** of the following situations:
   a) Prior to initiating treatment with azathioprine (AZA), mercaptopurine (6-MP) or thioguanine (6-TG).
   b) For individuals on thiopurine therapy with abnormal complete blood count (CBC) results that do not respond to dose reduction.

2) For individuals with inflammatory bowel disease, monitoring of thiopurine metabolite levels **MEETS COVERAGE CRITERIA** for **any** of the following indications:
   a) To measure blood levels in individuals suspected of having toxic responses to AZA and/or 6-MP (e.g., hepatotoxicity or myelotoxicity).
   b) To measure drug levels in individuals who have not responded to therapy (e.g., persistent fever, further weight loss, and bloody diarrhea).

3) For individuals with acute lymphoblastic leukemia, monitoring of thiopurine metabolite levels **MEETS COVERAGE CRITERIA** for **any** of the following situations:
   a) For individuals showing signs of a lack of myelosuppression while on therapy.
   b) For individuals who do not tolerate thiopurines.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

4) For all other situations not addressed above, phenotypic analysis of the enzyme TPMT **DOES NOT MEET COVERAGE CRITERIA**.

5) For all other situations not addressed above, analysis of the metabolite markers of azathioprine and 6-mercaptopurine, including 6-methyl-mercaptopurine ribonucleotides (6-MMRP) and 6-thioguanine nucleotides (6-TGN), **DOES NOT MEET COVERAGE CRITERIA**.

Table of Terminology

Term	Definition
6-MMRP	6-methyl-mercaptopurine ribonucleotides
6-MMP	6-methyl-mercaptopurine
6-MP	6-mercaptopurine
6-TG	6-thioguanine
6-TGN	6-thioguanine nucleotides
ACG	American College of Gastroenterology

AGA	American Gastroenterological Association
ASC	Acute severe colitis
ALL	Acute lymphoblastic leukaemia
AZA	Azathioprine
BNF	British National Formulary
BNFC	British National Formulary for Children
BSG	British Society of Gastroenterology
CBC	Complete blood count
CLIA ’88	Clinical Laboratory Improvement Amendments Of 1988
CMS	Centers for Medicare and Medicaid Services
CPIC	Clinical Pharmacogenetics Implementation Consortium
ECCO	European Crohn's and Colitis Organization
ESPGHAN	European Society of Paediatric Gastroenterology, Hepatology and Nutrition
FDA	Food and Drug Administration
HPLC	High-performance liquid chromatography
IBD	Inflammatory bowel disease
IVCS	Intravenous corticosteroids
LCAs	Local coverage articles
LCDs	Local coverage determinations
LDTs	Laboratory-developed tests
LFTs	Liver function tests
NASPGHAN	North American Society for Pediatric Gastroenterology, Hepatology and Nutrition
NCCN	National Comprehensive Cancer Network
NICE	National Institute for Health and Care Excellence
NRH	Nodular regenerative hyperplasia
NUDT15	Nudix hydrolase 15
RBC	Red blood count
RCTs	Randomized controlled trials
SFR	Steroid-free clinical remission
TDM	Therapeutic drug monitoring
TIL	Thiopurine-induced leukopenia
TPMT	Thiopurine methyltransferase
UC	Ulcerative colitis

Scientific Background

The thiopurine drugs 6-mercaptopurine (6-MP), azathioprine (AZA), and thioguanine remain a mainstay of immunomodulator therapy (Belmont, 2022; Rubin, 2022; Tantisira, 2023). However, several metabolites of these drugs (particularly 6-thioguanine [6-TG] and 6-methylmercaptopurine [6-MMP]) have been associated with harmful side effects, such as lowered therapeutic efficacy, hepatotoxicity, bone marrow toxicity, and more. The management of these toxic metabolites is further complicated by the many polymorphisms (and therefore efficacy in metabolism) of the genes responsible for metabolizing these drugs. Due to these toxic side effects, there has been significant investigation on

monitoring of these metabolites to identify the optimal dose of a thiopurine for an individual patient. This process is called therapeutic drug monitoring (TDM) (Rubin, 2022).

Two enzymes are responsible for catalyzing these reactions: thiopurine methyltransferase (TPMT) and hypoxanthine phosphoribosyl transferase. TPMT enzyme activity is a major factor determining AZA and 6-MP metabolism, and therefore 6-TG and 6-MMP levels. The majority of the population has wild type *TPMT* and normal enzyme activity, while 11% are heterozygous and have corresponding low TPMT enzyme activity, and 0.3% (1 in 300) have negligible activity (Lennard et al., 1993; Lennard et al., 1989; Rubin, 2022). Intermediate and normal metabolizers can have up to a threefold difference in initial target doses of AZA and 6-MP to achieve therapeutic 6-TG concentrations (Gardiner et al., 2008). Measurement of TPMT enzyme activity before instituting AZA or 6-MP may help prevent toxicity by identifying individuals with low or absent TPMT enzyme activity as well as identify those with higher than average TPMT activity who may remain refractory to conventional dosages (Rubin, 2022). Dosing strategies involving such testing may be cost-effective (Cuffari et al., 2004; Dubinsky et al., 2005; Winter et al., 2004). However, prediction of toxicity is not consistently reliable, as other enzymes are also likely to play a role in determining toxicity, such as glutathione-S-transferase (Stocco et al., 2007), and drug interactions must be taken into account (Dewit et al., 2002; Gilissen et al., 2005; Szumlanski & Weinshilboum, 1995). Thus, even though TPMT testing is recommended, a complete blood count (CBC), and also liver function tests, must still be obtained (Belmont, 2022; Relling et al., 2019).

Another enzyme that may contribute to thiopurine metabolism is nucleoside diphosphate-linked moiety X motif 15, NUDIX 15 (NUDT15). Variants in this enzyme's genotype and subsequent phenotype may lead to drastically reduced tolerance of 6-MP (Tantisira, 2023). Moriyama et al. proposed that NUDT15 variants cause thiopurines' mechanism of action to fail by preventing the thiopurine metabolites' incorporation into DNA. This causes these metabolites to remain active and therefore toxic (Moriyama et al., 2016). The frequency of these NUDT15 variants varies across populations, with the "poor metabolizer" phenotype reaching as high as 1 in 50 in East Asian populations. Despite the data indicating NUDT15's role in thiopurine toxicity, guidelines for its assessment have not reached a consensus, and expert opinions and practices are mixed (Tantisira, 2023).

Therapeutic drug monitoring (TDM) is the measurement of serum, plasma, or urinary concentrations of a given drug. This can be measured in a variety of ways, including high performance liquid chromatography (HPLC) or mass spectrometry approaches such as GC-MS (Eadie, 2002). TDM is proposed to allow a clinician to identify the "optimal" dose of a drug (such as a thiopurine) for a patient, thereby maximizing therapeutic efficacy and minimizing harmful side effects. Non-TDM approaches typically involve starting at low doses, then adjusting if the patient is tolerating the drug well or poorly, whereas TDM takes a more proactive approach in managing dose (Rubin, 2022). Several studies have attempted to identify standardized ranges of "optimal" metabolite concentrations. For example, the optimal concentration of the 6-TGN metabolite was found to be between 230 and 400 picomoles per 8 x 10^8 erythrocytes by Dubinsky et al. In that same study, bone marrow toxicity was found to correlate with levels above 400 picomoles per 8 x 10^8 erythrocytes (Dubinsky et al., 2000). Although there are potential limitations to TDM for thiopurines (such as intra-individual variability, lack of correlation with toxicity for 6-MMP, and so on), TDM used in conjunction with TPMT and NUDT15 assessment may allow clinicians to increase the therapeutic efficacy of thiopurines (Rubin, 2022; Tantisira, 2023).

Clinical Utility and Validity

Haines et al. (2011) performed a retrospective study of the utility of measuring thiopurine metabolites in "inadequately controlled" inflammatory bowel disease (IBD). 63 patients with IBD were included, and

these patients were treated with AZA or 6-mercaptopurine. On weight-based criteria, 50% patients were underdosed. However, metabolite study suggested that "7 (11%) patients were noncompliant, 18 (29%) were being underdosed, 33 (52%) were refractory to treatment with either appropriate (41%) or elevated (11%) metabolite concentrations, and 6 (10%) had a raised 6-methyl mercaptopurine: 6-thioguanine nucleotide ratio consistent with aberrant thiopurine metabolism". The clinical outcome of 87% of patients improved when the treatment was shifted to a metabolite-based algorithm, whereas 3 of 17 patients improved when the discordant action was taken. The authors concluded that "Thiopurine metabolite testing is a potentially powerful tool for optimizing thiopurine usage in IBD" (Haines et al., 2011).

Lee et al. (2017) evaluated 165 patients undergoing thiopurine treatment for Crohn's Disease. Thiopurine metabolite levels were measured, and both *TPMT* and *NUDT15* were genotyped. The authors found 95 patients responded to treatment whereas 45 did not. The median 6-TGN (the primary metabolite of 6-thioguanine) was significantly higher in responders than nonresponders. At a 6-TGN level of 230 pmol/8 x 10^8 blood cells, the odds ratio was 4.63 for responders to nonresponders. *NUDT15* variants were also found to be associated with "severe, early, leukopenia" with an average reduction of 88.2% from baseline white blood cell count at 4 weeks. The authors concluded that their findings "support the role of therapeutic drug monitoring in thiopurine maintenance treatment to optimize thiopurine therapy, especially, for non-responding CD patients" (Lee et al., 2017).

Spencer et al. (2019) compared "standard" and "optimized" thiopurine dosing regimens in 216 pediatric IBD patients. The "optimal" level was decided at "6-TGN >235 pmol/8 × 10^8 RBC", and the metabolite levels were correlated between the primary outcome of "steroid-free clinical remission (SFR)". Both groups were found to have similar initial and 6-month metabolite levels. SFR was achieved in 74% of the 180 patients on thiopurines at 6 months. The authors concluded that "steroid-free clinical remission and 6-TGN levels at 6 months were no different between a standardized, fixed dosing strategy and a metabolite-driven, optimized dosing strategy" (Spencer et al., 2019).

Meijer et al. (2017) evaluated the effects of thiopurine metabolites on clinical signs and if patient characteristics affected metabolite generation. 940 "laboratory findings" from 424 patients were examined. 6-TGN (a metabolite of azathioprine [AZA] and mercaptopurine) was found to negatively correlate with RBC count, WBC count, and neutrophil count. However, in patients using 6-thioguanine, those 6-TGN concentrations correlated positively with WBC count. An inverse correlation was observed between age and 6-TGN concentrations in AZA or 6-thioguanine patients, as well as between body mass index and 6-TGN in AZA or mercaptopurine patients. The authors concluded that "thiopurine derivative therapy influenced bone marrow production and the size of red blood cells. Age and body mass index were important pharmacokinetic factors in the generation of 6-TGN" (Meijer et al., 2017).

Estevinho et al. (2017) performed a meta-analysis to "assess the clinical value of 6-thioguanine nucleotide thresholds; and ii] to compare mean 6-thioguanine nucleotide concentrations between patients in clinical remission vs. those with active disease." A total of 22 records were used in cut-off comparisons and 12 were used in the 6-thioguanine nucleotide mean differences analysis. The authors calculated the global odds ratio for remission in patients with 6-thioguanine nucleotides above predefined thresholds to be 3.95. The authors also found an odds ratio for remission of 2.25 for the 235 pmol/8 x 10^8 RBC threshold, and an odds ratio of 4.71 for the 250 pmol /8 x 10^8 RBC threshold. Finally, the authors found a "pooled difference" of 63.37 pmol/8 x 10^8 RBC between patients in clinical remission and those not in remission. Overall, the authors concluded that the study reinforced the link between and 6-thioguanine nucleotide levels and clinical remission in inflammatory bowel diseases (Estevinho et al., 2017).

Toksvang et al. (2019) performed a meta-analysis focusing on "incidence of hepatotoxicity in patients [with childhood acute lymphoblastic leukaemia, ALL or inflammatory bowel disease, IBD] treated with 6TG [6-thioguanine]". 42 reports were included, further broken down into "four randomised controlled trials (RCTs) including 3,993 patients, 20 observational studies including 796 patients, and 18 case reports including 60 patients". The authors measured hepatotoxicity by "sinusoidal obstruction syndrome", which occurred in 9-25% of ALL patients in two of the four RCTs at a dosage of 40–60 mg/m^2/day. The authors also noted that at a dosage of 23 mg/m^2/day, nodular regenerative hyperplasia (NRH) occurred in 14% of IBD patients. At a dosage of 12 mg/m^2/day, NRH occurred in 6% of IBD patients, which was noted to be similar to background incidence. The authors therefore concluded that doses at or under 12 mg/m^2/day can "probably be considered safe" (Toksvang et al., 2019).

Zhu et al. (2019) evaluated the "predictive sensitivity based on 6TGN [6-thioguanine nucleotide] by subgrouping patients according to their *NUDT15* R139C genotypes". The authors included 411 patients with Crohn's Disease. Two subgroups of *NUDT15* genotypes were created, "CC" (n = 342) and "CT" (n = 65), with the final four patients harboring a TT genotype. Thiopurine-induced leukopenia (TIL) was the primary clinical endpoint measured. The authors found that of the 342 patients with a CC genotype, only 35 developed TIL (10.2%), but of the 65 CT patients, 33 developed TIL (50.2%). All four of the TT patients developed TIL. The authors also found that in both CC and CT genotypes, the median 6TGH level was higher in patients with TIL than patients without TIL (for CC, 474.8 pmol/8 × 10^8 RBC vs 306.0 pmol/8 × 10^8 RBC, for CT 291.7 / 8 × 10^8 RBC vs 217.6/8 × 10^8 RBC). From this data, the authors calculated the "cut-off" (a threshold to identify an optimal number of cases) of the CT genotype to be 319.2 pmol/8 × 10^8 RBC and the cut-off for CC to be 411.5 pmol/8 × 10^8 RBC). Overall, the authors concluded that "The predictive sensitivity of TIL based on 6TGN is dramatically increased after subgrouping according to *NUDT15* R139C genotypes. Applying 6TGN cut-off levels to adjust thiopurine therapies based on *NUDT15* is strongly recommended" (Zhu et al., 2019).

Guidelines and Recommendations

National Comprehensive Cancer Network (NCCN)

In version 2 of the 2023 guidelines for Pediatric Acute Lymphoblastic Leukemia, the NCCN recommends that "for patients homozygous for normal function TPMT and NUDT15, who do not appear to tolerate thiopurines, consider measuring erythrocyte thiopurine metabolites and/or erythrocyte TPMT activity. Genetic testing may fail to identify rare or previously undiscovered no function alleles." The NCCN also writes that "genetic testing for no function alleles of TPMT and NUDT15 should be considered prior to the initiation of thiopurine therapy" (NCCN, 2023b).

In version 1 of the 2023 guidelines for Acute Lymphoblastic Leukemia, the NCCN notes that "quantification of 6-MP metabolites can be very useful in determining whether lack of myelosuppression is due to non-adherence or hypermetabolism".

The 2023 guidelines also state, "for patients receiving 6-MP, consider testing for TPMT gene polymorphisms, particularly in patients who develop severe neutropenia after starting 6-MP. Testing for both TPMT and NUDT15 variant status should be considered, especially for patients of East Asian origin" (NCCN, 2023a).

Toronto Ulcerative Colitis Consensus Group/American College of Gastroenterology (ACG)

Bressler et al. (2015) published clinical practice guidelines for the medical management of non-

hospitalized ulcerative colitis on behalf of the Toronto Ulcerative Colitis Consensus Group, which reaffirmed recommendations from the American College of Gastroenterology, Practice Parameters Committee (Kornbluth & Sachar, 2010) for thiopurine therapy (Bressler et al., 2015). The authors stated that "…a TPMT assay is necessary before initiation of treatment to identify patients at risk for severe dose-dependent myelosuppression…therefore, thiopurine metabolite levels may be helpful to guide therapy. Note that TPMT testing does not replace the need for mandatory monitoring of complete blood cell count" (Bressler et al., 2015).

American College of Gastroenterology (ACG)

The ACG published a guideline for the "Management of Crohn's Disease in Adults". Their relevant recommendations include:

"Thiopurine methyltransferase (TPMT) testing should be considered before initial use of azathioprine or 6-mercaptopurine to treat patients with Crohn's disease (strong recommendation, low level of evidence)" (Lichtenstein et al., 2018).

The ACG also published a guideline for ulcerative colitis (UC) in adults. Their relevant recommendations include:

"The patient with nonresponse or loss of response to therapy should be assessed with therapeutic drug monitoring to identify the reason for lack of response and whether to optimize the existing therapy or to select an alternate therapy."

"There is insufficient evidence supporting a benefit for proactive therapeutic drug monitoring in all unselected patients with UC in remission" (Rubin et al., 2019).

North American Society for Pediatric Gastroenterology, Hepatology and Nutrition (NASPGHAN) Committee

In 2013, the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition (NASPGHAN) Committee on Inflammatory Bowel Disease published consensus recommendations on the role of TPMT and thiopurine metabolite testing in pediatric IBD The recommendations included the following (Benkov et al., 2013):

- "TPMT testing is recommended before initiation of TPs to identify individuals who are homozygote recessive or have extremely low TPMT activity, with the latter having more reliability than the former. (HIGH)."
- "Individuals who are homozygous recessive or have extremely low TPMT activity should avoid use of TPs because of concerns for significant leukopenia. (HIGH)"
- "TPMT testing does not predict all cases of leukopenia and has no value to predict hypersensitivity adverse effects such as pancreatitis. Any potential value to reduce the risk of malignancy has not been studied. All individuals on TPs should have routine monitoring with CBC and WBC count differential to evaluate for leukopenia regardless of TPMT testing results. (HIGH)"
- "Metabolite testing can be used to determine adherence to TP therapy. (HIGH)"
- "Metabolite testing can be used to guide dose increases or modifications in patients with active disease. Consideration would include either increasing the dose, changing therapy or for those with elevated transaminases or an elevated 6-MMP, using adjunctive allopurinol to help raise 6-TG metabolites and suppress formation of 6-MMP. (MODERATE)"

- "Routine and repetitive metabolite testing has little or no role in patients who are doing well and taking an acceptable dose of a TP. (MODERATE)"

American Gastroenterological Association (AGA)

In 2017, the AGA published guidelines on Therapeutic Drug Monitoring in Inflammatory Bowel Disease which recommend (Feuerstein et al., 2017):

- "In adult patients treated with thiopurines with active IBD or adverse effects thought to be due to thiopurine toxicity, the AGA suggests reactive thiopurine metabolite monitoring to guide treatment changes."
- "In adult patients with quiescent IBD treated with thiopurines, the AGA suggests against routine thiopurine metabolite monitoring."

The AGA published an Institute Technical Review on the Role of Therapeutic Drug Monitoring in the Management of Inflammatory Bowel Diseases in the same year. In it, they note that IBD patients treated with thiopurines may benefit from reactive TDM to guide treatment changes (Vande Casteele et al., 2017).

In the 2020 AGA guidelines for "Management of Moderate to Severe Ulcerative Colitis", the AGA remarks that "therapeutic drug monitoring to guide the use of biologic therapy has been addressed in separate AGA guidelines". The "separate AGA guidelines" refer to the 2017 edition above (Feuerstein et al., 2020).

Clinical Pharmacogenetics Implementation Consortium (CPIC)

In their guideline for "Thiopurine Dosing Based on TPMT and NUDT15 Genotypes", CPIC notes that "mercaptopurine and azathioprine are generally used for nonmalignant immunologic disorders, mercaptopurine for lymphoid malignancies, and thioguanine for myeloid leukemias". However, CPIC also writes that "variants in NUDT15 have been identified that strongly influence thiopurine tolerance in patients with acute lymphoblastic leukemia (ALL) and those with inflammatory bowel diseases" (Relling et al., 2019).

In 2020, the authors of this guideline added recommendations for TPMT and NUDT15 indeterminate phenotypes. That update is as follows:

- "For TPMT and NUDT15 indeterminate phenotypes, (i.e. combination of uncertain and/or unknown function alleles):
    - TPMT indeterminate: Consider evaluating TPMT erythrocyte activity to assess TPMT phenotype.
    - NUDT15 indeterminate: If thiopurines are required and NUDT15 status is unknown, monitor closely for toxicity" (CPIC, 2020).

British Society of Gastroenterology (BSG)

The BSG published "consensus guidelines" on management of inflammatory bowel disease in adults. They recommend checking TPMT status in "all patients considered for thiopurine therapy". They also recommend testing the NUDT15 genotype if "available".

The BSG also writes that thiopurine metabolites should be checked if a patient experiences myelotoxicity

as a side effect. Similarly, if a patient demonstrates "newly abnormal LFTs [liver function tests]", thiopurine metabolites should be checked. Also, BSG states that "all IBD patients considered for thiopurine therapy should have assessment of thiopurine methyltransferase (TPMT) status" (Lamb et al., 2019).

Overall, the BSG writes that thiopurine metabolites can be used to "optimize drug dosing" and "suggest that metabolite monitoring may be used for those with inadequate response to therapy or toxicity, but should not be a substitute for routine monitoring blood tests" (Lamb et al., 2019).

Canadian Association of Gastroenterology (CAG)

The Canadian Association of Gastroenterology published a guideline on "the Medical Management of Pediatric Luminal Crohn's Disease." The guidelines "suggested that testing for TPMT by genotype or enzymatic activity be done prior to initiating thiopurine therapy to guide dosing" (Mack et al., 2019).

An additional guideline for [Adult] Luminal Crohn's Disease specifies "because some patients may have low or absent levels of the enzyme (thiopurine methyltransferase (TPMT) needed to metabolize thiopurines, a TPMT assay should be performed before initiation of treatment to identify patients at risk for severe toxicity" (Panaccione et al., 2018).

National Institute for Health and Care Excellence (NICE)

NICE, released guidelines on Crohn's Disease Management in 2019. In it, they recommend to "Monitor the effects of azathioprine, mercaptopurine, and methotrexate as advised in the British national formulary (BNF) or British national formulary for children (BNFC)" (NICE, 2019).

European Crohn's and Colitis Organization and European Society of Paediatric Gastroenterology, Hepatology and Nutrition (ECCO and ESPGHAN)

These joint guidelines note that "measuring…6-TG and 6-MMP levels after 2–3 months, may aid in optimizing thiopurine dosing." Measuring thiopurine metabolites is recommended in the following scenarios:

- In patients with incomplete response on stable thiopurine dosage
- In patients who present with leucopenia or elevated transaminases
- After acute severe colitis (ASC) responsive to intravenous corticosteroids (IVCS)
- When poor compliance is suspected (Turner et al., 2018).

Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
80299	Quantitation of therapeutic drug, not elsewhere specified
82657	Enzyme activity in blood cells, cultured cells, or tissue, not elsewhere specified; nonradioactive substrate, each specimen

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

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Revision History

Revision Date	Summary of Changes
09/06/2023	Reviewed and Updated: Updated the background, guidelines and recommendations, and evidence-based scientific references. Literature review did not necessitate any modifications to coverage criteria. The following edits were made for clarity: All CC edited for clarity and consistency, including replacing “patient” with “individual”

Nerve Fiber Density Testing

Policy Number: AHS – M2112 – Nerve Fiber Density Testing	Prior Policy Name and Number, as applicable:
Initial Presentation Date: 09/18/2015 Revision Date: February 1, 2025	

Policy Description

Nerve fiber density testing involves analysis of skin biopsy stained with an antibody to antiprotein gene product 9.5 (Wilkinson et al., 1989) which avidly stains all axons (Dalsgaard et al., 1989). The number and morphology of axons within the epidermis are evaluated to determine epidermal nerve fiber density (McCarthy et al., 1995) and assess for the presence and degree of neuropathy (Smith & Gibson, 2022).

Related Policies

Policy Number	Policy Title
N/A	

Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) For the diagnosis of small-fiber neuropathy, epidermal nerve fiber density measurement from a skin biopsy **MEETS COVERAGE CRITERIA** when **all** of the following conditions are met:
   a) An individual presents with symptoms of painful sensory neuropathy;
   b) There is no history of a disorder known to predispose to painful neuropathy (e.g., diabetic neuropathy, toxic neuropathy, HIV neuropathy, celiac neuropathy, inherited neuropathy);

c) Physical examination shows no evidence of findings consistent with large-fiber neuropathy, such as reduced or absent muscle-stretch reflexes or reduced proprioception and vibration sensation;

d) Electromyography and nerve-conduction studies are normal and show no evidence of large-fiber neuropathy.

2) For all other situations not described above, epidermal nerve fiber density measurement from a skin biopsy **DOES NOT MEET COVERAGE CRITERIA**.

3) Measurement of sweat gland nerve fiber density **DOES NOT MEET COVERAGE CRITERIA**.

Table of Terminology

Term	Definition
AACE	American Association of Clinical Endocrinologists
AAN	American Academy of Neurology
AANEM	American Association of Neuromuscular and Electrodiagnostic Medicine
AAPM&R	American Academy of Physical Medicine and Rehabilitation
ACE	American College of Endocrinology
ADA	American Diabetes Association
BAEPs	Brainstem auditory evoked potentials
CCM	Corneal confocal microscopy
CIDP	Chronic inflammatory demyelinating polyneuropathy
CMS	Centers for Medicare and Medicaid Services
CMT1A	*Charcot-Marie-Tooth Disease Type 1A*
CNBD	Corneal nerve branch density
CNFD	Corneal nerve fiber density
CNFL	Corneal nerve fiber length
CTBD	Corneal total branch density
DNFL	Dermal nerve fiber length
DSP	Distal symmetric polyneuropathy
DSPN	Diabetes and neuropathy
EDS	Ehlers-Danlos Syndrome
EFNS	European Federation of Neurological Societies
ENFD	Epidermal nerve fiber density
FAP	Familial amyloid polyneuropathy
FD	Fabry disease
FM	Fibromyalgia
FMS	Fibromyalgia syndrome
FRDA	Friedreich's ataxia
H&E	Haematoxylin and eosin
HIV	Human immunodeficiency virus
IASP	International Association for the Study of Pain
IENF	Intraepidermal nerve fiber

IENFD	Intraepidermal nerve fiber density
IETNFL	Intraepidermal total nerve fiber length
IGT	Impaired glucose tolerance
IHC	Immunohistochemistry
IMMPACT	Initiative on Methods, Measurement, and Pain Assessment in Clinical Trials
MAL	Mean axonal length
MP	Medial plantar
NCS	Nerve conduction studies
NeuPSIG	Neuropathic Pain Special Interest Group
NIS-LL	Neuropathy Impairment Score in the Lower Limb
OH	Overt hypothyroidism
PD	Parkinson's Disease
PGP	Protein gene product
PNS	Peripheral Nerve Society
Product 9.5	Protein gene product 9.5
QST	Quantitative sensory testing
ROC	Receiver-operating characteristic
SENPD	Subepidermal nerve plexus densities
SFN	Small fiber neuropathy
SFSG	Small-fiber sensory ganglionopathy
SFSN	Small fiber sensory neuropathy
SFSPN	Small fiber sensory polyneuropathy
SGII	Sweat gland innervation index
SGNF	Sweat gland nerve fiber
SH	Subclinical hypothyroidism
T1DM	Type 1 diabetes without neuropathy
VAS	Visual analog scale
VEPs	Visual evoked potentials
VIP	Vasoactive intestinal peptide

Scientific Background

Neuropathy can be defined as dysfunction of the peripheral nerves, leading to weakness or a numbness feeling in the hands, feet, arms, or legs. This disorder can be caused by several ailments including infections, traumatic injuries, and metabolic problems such as diabetes. As the pathology of neuropathy is usually first evident in nerve terminals; both sensory and autonomic nerves have terminals in the epidermis of the skin (Chien et al., 2001), evaluation of nerve fibers in skin biopsy is a reasonable approach to the diagnosis of neuropathy. Skin biopsy is a commonly used technique for assessment of peripheral nerve disease. The biopsy is a benign procedure with few and reasonably tolerated side effects. Multiple biopsies can be performed without issue. The skin tissue is obtained with a 3 mm "punch," which is then cut into thick sections. These segments are stained with antiprotein gene product 9.5 antibody (PGP 9.5), which stains all axons. The status of these axons is then evaluated to determine epidermal nerve density. The biopsy site depends on the specific indication; for example, a length-dependent peripheral neuropathy typically uses biopsies at the distal leg and a proximal site such as the

lateral thigh. Nerve fiber biopsy has numerous applications, such as differentiating between neurogenic and myopathic conditions, characterizing muscular disease, and evaluation of peripheral neuropathies. However, the most common use for skin biopsy is evaluation of small fiber sensory neuropathy (Smith & Gibson, 2022).

Many chronic disorders lead to small fiber peripheral neuropathy, including diabetes, thyroid dysfunction, sarcoidosis, vitamin B12 deficiency, human immunodeficiency virus (HIV), celiac disease, and paraneoplastic syndromes. Small fiber neuropathy is often a challenging clinical problem as patients commonly have severe complaints, but standard electrophysiologic testing is often normal; moreover, sural nerve biopsy may be normal or only minimally abnormal. The range of applications of skin biopsy has been expanded to include autonomic neuropathies and immune-mediated and inherited demyelinating neuropathies (Lauria & Devigili, 2007). However, skin biopsy is not useful in assessment of the etiology of neuropathy. Skin biopsy cannot replace nerve biopsy when neuropathological examination of mixed or large-fiber neuropathy is needed or when a vasculitis pathogenesis is suspected (Lauria & Devigili, 2007).

Proprietary Testing

The assessment of epidermal nerve fiber (ENFD) and sweat gland nerve fiber (SGNF) density with PGP 9.5, for the evaluation of small fiber neuropathy, is commercially available from Therapath with a biopsy kit (Therapath, 2022) and from BakoDx with a biopsy kit that also provides an assessment of SFN's degree of severity. BakoDx's specificity of ENFD is 95%-97%; and the sensitivity is approximately 90% (BakoDx, 2022). Intraepidermal nerve fiber (IENF)-density measurement may also be performed with proprietary tests done by local research pathology labs. Ipsum Diagnostics developed a ENFD test that uses H&E as the background stain opposed to the IHC background stain that is regularly implemented by other labs (Ipsum Diagnostics, 2022). Additional labs, such as Corinthian Reference Lab, also offer commercial ENFD tests kits to physicians to aid in a diagnosis of small fiber neuropathy (CRL, 2022; NeuroPath, 2022).

Clinical Utility and Validity

A committee consisting of the American Academy of Neurology (AAN), American Association of Neuromuscular and Electrodiagnostic Medicine (AANEM) and the American Academy of Physical Medicine and Rehabilitation (AAPM&R) performed a literature review to evaluate the diagnostic accuracy of intraepidermal nerve fiber (IENF) density in the detection of small fiber neuropathy. A total of 106 articles were reviewed (England et al., 2009b).

The committee noted that all the case control studies showed a significant reduction in IENF density in polyneuropathy patients compared to controls. The sensitivity of decreased IENF density for the diagnosis of polyneuropathy ranged from 45% to 90%. The specificity of normal IENF density for the absence of polyneuropathy ranged from 95% to 97%. The committee suggested that the absence of reduced IENF density (using the clinical impression as the diagnostic reference standard) would not "rule out" polyneuropathy, but reduced IENF density would raise the likelihood of polyneuropathy (England et al., 2009b).

The authors also assessed the sensitivity of IENF density assessment at the ankle. Four studies were identified. In these studies, the specificity of the test ranged from 95% to 97.5%, and the sensitivities ranged from 24% to 100%. This study found that "among patients with symptoms of SFSN [small fiber sensory neuropathy] and an abnormal pinprick examination in the feet, but normal ankle reflexes,

normal vibration sensibility, and normal NCS [nerve conduction studies], an IENF density of <8 fibers/mm at the dorsal foot provided a sensitivity of 88%, a specificity of 91%, a positive predictive value of 0.9, and a negative predictive value of 0.83 for the diagnosis of SFSN" (England et al., 2009b). The committee concluded that "IENF density assessment using PGP 9.5 immunohistochemistry is a validated, reproducible marker of small fiber sensory pathology. Skin biopsy with IENF density assessment is possibly useful to identify DSP [distal symmetric polyneuropathy] which includes SFSN in symptomatic patients with suspected polyneuropathy (Class III)" (England et al., 2009b).

Collongues et al. (2018) created a normative dataset for intraepidermal nerve fibers from the distal leg. Three hundred healthy controls contributed samples. The authors measured nerve density with protein gene product-9.5 immunocytochemistry and brightfield microscopy. The fifth percentile of intraepidermal nerve fiber density was calculated to be "7.6156-0.0769 x age (years) + 1.5506 x gender (woman = 1; man = 0)" (Collongues et al., 2018).

Piscosquito et al. (2021) studied how understanding nerve fiber spatial distribution could help improve the diagnostic yield of skin biopsy. The study included 31 patients with SFN symptoms, normal nerve conduction study, abnormal quantitative sensory testing, and normal IENF density, 31 healthy controls, and 31 SFN patients with reduced IENF density. The distance between consecutive IENFs in the three groups was measured. It was found that the mean interfiber distances did not differ between patients with normal counts and healthy controls. An inter-fiber distance of 350 um was identified "as the measure that better differentiated patients from controls (AUC = 0.85, sensitivity: 74%, specificity: 94%)." The authors conclude that "the presence of a stretch of denervated epidermis longer than 350 μm is a parameter able to increase the diagnostic efficiency of skin biopsy" (Piscosquito et al., 2021).

Corrà et al. (2021) have developed an automated method of IENFD determination aiming to improve diagnostic accuracy and applicability in clinical practice. IENFD generally requires manual analysis by one to three operators, but the automated method requires reduced operator count. The authors studied 60 skin biopsy specimens stained with PGP 9.5. IENFD was first determined manually by three operators, then automatically. The automated method resulted in less variability and similarly high reliability compared to the manual method. The automated method took 15 seconds; the manual method took 10 minutes. The authors conclude that "this automated method rapidly and reliably detects small nerve fibers in skin biopsies with clear advantages over the classical manual technique" (Corrà et al., 2021)

Sensory Neuropathy

McArthur et al. (1998) established the normative reference range and diagnostic efficiency of nerve fiber density testing for sensory neuropathies in 98 normal controls and 20 patients with sensory neuropathies. The density of intraepidermal fibers in normal controls was found to be 21.4 ± 10.4 per mm in the thigh with the fifth percentile to be 5.2/mm. Density of normal controls in the leg was found to be 13.8±6.7 per mm with the fifth percentile to be 3.8/mm. Using the fifth percentile for the leg as a cutoff, the technique had a "positive predictive value of 75%, a negative predictive value of 90%, and a diagnostic efficiency of 88%" (McArthur et al., 1998).

Chien et al. (2001) evaluated skin biopsy specimens from the distal leg and distal forearm of 55 healthy controls and 35 patients with sensory neuropathy. In the healthy controls, conventional IENF densities in the distal forearm and in the distal leg were correlated (r=0.55) with significantly higher values in the distal forearm than in the distal leg (17.07±6.51 verses 12.92±5.33 fibers/mm). Compared to IENF densities of healthy controls, these values of neuropathic patients were significantly reduced in the distal

forearm (5.82±6.50 fibers/mm) and in the distal leg (2.40±2.30). The specificity of the test was found to be 95% (Chien et al., 2001).

Devigili et al. (2008) screened 486 patients and collected samples from 124 patients with sensory neuropathy. Among them, they identified 67 patients with pure small fiber neuropathy (SFN) using a new diagnostic "gold standard" based on the presence of at least two abnormal results after clinical examination, quantitative sensory testing (QST), and skin biopsy examination. They found that "Skin biopsy showed a diagnostic efficiency of 88.4%, clinical examination of 54.6% and QST of 46.9%. Receiver operating characteristic curve analysis confirmed the significantly higher performance of skin biopsy comparing with QST" (Devigili et al., 2008).

Devigili et al. (2019) also screened 150 patients previously diagnosed with sensory neuropathy and 352 new patients with suspected sensory neuropathy to establish diagnostic criteria for small fiber neuropathy. The diagnostic criteria were based on both QST and intraepidermal nerve fiber density (IENFD) measurements. Of the 352 new patients, small fiber neuropathy was diagnosed in 149 "based on the combination between two clinical signs and abnormal QST and IENFD (69.1%), abnormal QST alone (5.4%), or abnormal IENFD alone (20.1%)" (Devigili et al., 2019). The authors noted that "The combination of clinical signs and abnormal QST and/or IENFD findings can more reliably lead to the diagnosis of small fibre neuropathy than the combination of abnormal QST and IENFD findings in the absence of clinical signs" (Devigili et al., 2019). Further, sensory symptoms alone were not a reliable screening method for sensory neuropathy in this study.

Vlckova-Moravcova et al. (2008) measured IENF densities and subepidermal nerve plexus densities (SENPD) quantified by immunostaining in skin punch biopsies. Samples were taken from the distal calf in 99 patients with clinical symptoms of painful sensory neuropathy; samples were also taken from 37 age-matched healthy volunteers. They found that "In patients with neuropathy, IENFD and SENPD were reduced to about 50% of controls. Using receiver-operating characteristic (ROC) curve analysis of IENFD values, the diagnostic sensitivity for detecting neuropathy was 0.80 and the specificity 0.82. For SENPD, sensitivity was 0.81 and specificity 0.88. With ROC analysis of both IENFD and SENPD together, the diagnostic sensitivity was further improved to 0.92" (Vlckova-Moravcova et al., 2008). The authors concluded that "the combined examination of IENFD and SENPD is a highly sensitive and specific diagnostic tool in patients suspected to suffer from painful sensory neuropathies but with normal values on clinical neurophysiological studies" (Vlckova-Moravcova et al., 2008).

Gibbons et al. (2006) studied 28 patients with "sensory complaints of unknown etiology." Each patient had repeated skin biopsies. Patients with large nerve fiber swellings on initial biopsy showed a decline in epidermal nerve fiber density on repeated biopsies whereas patients without nerve fiber swellings did not have changes in nerve fiber density between biopsies. Patients with large nerve fiber swellings were most likely to present clinically with paresthesia (Gibbons et al., 2006).

Autonomic Neuropathy

Gibbons et al. (2009) developed a new technique to quantify the sweat gland nerve fiber density (SGNFD) using tissue prepared for the standard analysis of IENFD. The technique "differentiates groups of patients with mild diabetic neuropathy from healthy control subjects and correlates with both physical examination scores and symptoms relevant to sudomotor dysfunction"; further, this technique is proposed to provide a "reliable structural measure of sweat gland innervation that complements the investigation of small fiber neuropathies" (Gibbons et al., 2009). The authors validated the technique in 30 diabetic and 64 healthy subjects. Diabetic subjects had reduced SGNFD compared to controls at the

distal leg, distal thigh, and proximal thigh. The SGNFD at the distal leg of diabetic subjects decreased as the Neuropathy Impairment Score in the lower limb (NIS-LL) worsened (r = -0.89) and was concordant with symptoms of reduced sweat production.

Luo et al. (2011) developed an alternative staining system using PGP 9.5 and counterstaining with Congo red which reduced the variations in measurements of sweat gland areas compared to the commonly used method by ~5.6-fold (2.47% ± 2.54% vs 13.97% ± 14.24%). The authors examined 35 diabetic patients and compared these results to controls. Diabetic patients had lower sweat gland innervation index (SGII) values than age- and sex-matched controls (2.60% ± 1.96% vs 4.84% ± 1.51%). The SGII values were lower in patients with anhidrosis of the feet versus those with normal sweating of the feet (0.89% ± 0.71% vs 3.10% ± 1.94%). The authors concluded that "skin biopsy offers combined assessment of sudomotor innervation" (Luo et al., 2011).

Diabetic Neuropathy

Those with both diabetes and metabolic syndrome have double the risk of peripheral neuropathy (Hovaguimian & Gibbons, 2011), and the prevalence of polyneuropathy is high in obese individuals, even those with normoglycemia (Callaghan et al., 2016). Diabetes and obesity are common metabolic drivers of peripheral neuropathy (Callaghan et al., 2018).

Alam et al. (2017) compared the diagnostic capability of corneal confocal microscopy (CCM) against a range of established measures of nerve damage in patients with diabetic neuropathy. Thirty patients with Type 1 diabetes without neuropathy (T1DM), 31 patients with Type 1 diabetes and neuropathy (DSPN), and 27 healthy controls underwent CCM, as well as QST, electrophysiology, and skin biopsy. Intra-epidermal nerve fiber density was found to have a diagnostic sensitivity of 0.61, specificity of 0.80, and area under the ROC curve of 0.73 (Alam et al., 2017).

Wang et al. (2021) studied the diagnostic utility of corneal confocal microscopy in type 2 diabetes peripheral neuropathy. 172 patients with Type 2 DM and 48 healthy patients were enrolled in the study and assessed for neurological symptoms and corneal nerve fiber density was measured. "Corneal nerve fiber density, corneal nerve fiber length and corneal nerve branch density were significantly reduced in patients with type 2 diabetes mellitus compared with normal healthy control subjects" (Wang et al., 2021). Cut-off values for corneal nerve fiber density (24.68), corneal nerve branch density (39), and corneal nerve fiber length (15.315) were determined. The authors state that corneal confocal microscopy can be applied to diagnose type 2 diabetes peripheral neuropathy; however, the cost of the equipment is expensive which hinders its large-scale clinical application (Wang et al., 2021).

Familial Amyloid Polyneuropathy (FAP)

Chao et al. (2015) investigated the "the pathology and clinical significance of sudomotor denervation." Skin biopsies of 28 familial amyloid polyneuropathy (FAP) patients were stained with two markers: protein gene product 9.5 (PGP 9.5) and vasoactive intestinal peptide (VIP) followed by quantitation according to SGII for PGP 9.5 (SGIIPGP 9.5) and VIP (SGIIVIP). The researchers found that "The SGIIPGP 9.5 and SGIIVIP of FAP patients were significantly lower than those of age- and gender-matched controls. The reduction of SGIIVIP was more severe than that of SGIIPGP 9.5 (p=0.002). Patients with orthostatic hypotension or absent sympathetic skin response at palms were associated with lower SGIIPGP 9.5 (p = 0.019 and 0.002, respectively). SGIIPGP 9.5 was negatively correlated with the disability grade at the time of skin biopsy (p=0.004) and was positively correlated with the interval from the time of skin biopsy to the time of wheelchair usage (p=0.029)" (Chao et al., 2015). The authors documented

"the pathological evidence of sudomotor denervation in FAP. SGIIPGP 9.5 was functionally correlated with autonomic symptoms, autonomic tests, ambulation status, and progression of disability" (Chao et al., 2015).

Erythromelalgia

Mantyh et al. (2016) investigated the clinical utility of nerve fiber density testing for erythromelalgia in a retrospective study of 52 consecutive patients with erythromelalgia. Most patients were found to have "abnormalities on functional nerve testing," but less than 10% of patients had decreased epidermal nerve fiber density. The authors concluded that "Skin biopsy for evaluation of epidermal nerve fiber density is not useful in the diagnosis of erythromelalgia; instead, physicians may wish to focus on functional nerve testing, which more reliably identifies this disease" (Mantyh et al., 2016).

Fibromyalgia (FM)

Caro and Winter (2014) studied 41 consecutive patients with fibromyalgia (FM) and 47 controls to establish the prevalence of small fiber neuropathy (SFN) in FM. The authors found that the epidermal nerve fiber density (ENFD) of patients with FM was more than controls at the calf and thigh (calf: mean ± SD 5.8 ± 2.8 versus 7.4 ± 1.9; thigh 9.3 ± 3.2 versus 11.3 ± 2.0). Advanced age was insufficient to explain this finding. The authors suggested that "small fiber neuropathy is likely to contribute to the pain symptoms of FM; that pain in this disorder arises, in part, from a peripheral immune-mediated process; and that measurement of ENFD may be a useful clinical tool in FM" (Caro & Winter, 2014).

Lawson et al. (2018) sought to characterize and distinguish the subset of patients with both fibromyalgia and small fiber polyneuropathy in 155 FM patients. These FM patients completed a Short Form McGill Questionnaire and visual analog scale in addition to having skin biopsies, nerve conduction studies (NCS), and serologic testing. The authors found that "Sural and medial plantar (MP) response amplitudes correlated with epidermal nerve fiber density, with markers of metabolic syndrome being more prevalent in this subset of patients. Pain intensity and quality did not distinguish patients" (Lawson et al., 2018). The authors concluded that "the FM-SFSPN subset of patients may be identified through sural and MP sensory NCS and/or skin biopsy but cannot be identified by pain features and intensity" (Lawson et al., 2018).

Evdokimov et al. (2020) characterized dermal skin innervation in patients with fibromyalgia syndrome (FMS). 86 patients with FMS and 35 healthy patients were enrolled in the study and the skin was immunoreacted with antibodies against protein gene product 9.5, calcitonine gene-related peptide, substance P, CD31, and neurofilament 200 for small fiber subtypes. Skin sections were assessed on each patient and dermal nerve fiber length (DNFL) was assessed. In FMS patients, DNFL of fibers with vessel contact was found to be reduced compared to healthy individuals. Overall, the authors conclude that there were less dermal nerve fibers in contact with blood vessels in FMS patients than in controls, which suggests "the possibility of a relationship with impaired thermal tolerance commonly reported by FMS patients" (Evdokimov et al., 2020).

Ganglionopathy

Provitera et al. (2018) researched the role of skin biopsy in differentiating SFN from small-fiber sensory ganglionopathy (SFSG). Both thigh and leg IENF were studied from 314 participants with small-fiber pathology and 288 healthy controls. The researchers found that "The leg:thigh IENF density ratio was significantly ($P < 0.01$) lower in patients with length-dependent SFN (0.44 ± 0.23) compared with

patients with SFSG (0.68 ± 0.28)" (Provitera et al., 2018). Overall, measurement of the thigh and leg IENF ratio has shown clinical utility in differentiating diagnoses between SFSG and length-dependent SFN.

Hypothyroidism

Magri et al. (2010) evaluated 18 neurologically asymptomatic patients newly diagnosed with overt (OH) or subclinical hypothyroidism (SH) and 15 healthy controls. The density of innervation was measured. The authors found that "an abnormal IENF density consistent with SFN was found in 60% of patients with OH at the distal leg and in 20% at the proximal site with OH and in 25% of cases at the distal leg and in 12.5% of cases at the proximal thigh in patients with SH" (Magri et al., 2010). The authors suggested that a "considerable number of untreated hypothyroid patients may have preclinical asymptomatic small-fiber sensory neuropathy" (Magri et al., 2010).

Gupta et al. (2016) investigated the "electrophysiological alterations of some selected variables of nerve conduction, brainstem auditory evoked potentials (BAEPs), and visual evoked potentials (VEPs) in hypothyroid patients." Sixty patients with hypothyroidism and 60 controls had nerve conduction studies (including parameters as latencies, conduction velocities, and amplitude of motor and sensory nerves) performed. BAEPs and VEPs were also assessed. The authors found that on comparative evaluation, there was a significant increase in latency of median, ulnar, tibial, and sural nerves; the authors also found a decrease in conduction velocities of all the tested nerves and a decrease in amplitude of median, tibial, and sural nerves was observed in hypothyroid patients. The authors suggested that "peripheral and central neuropathy develops in patients of hypothyroidism at an early stage of disease and the electrophysiological investigations of such patients can help in timely detection and treatment of neurological disorders that occur due to thyroid hormone deficiency" (Gupta et al., 2016).

Fabry Disease (FD)

About 80% of patients with Fabry disease (FD) suffer from painful neuropathy; neuropathic pain in FD is associated with SFN. Torvin Moller et al. (2009) explored the frequency of symptoms and the functional and structural involvement of the nervous system in female patients by examining the presence of pain, manifestations of peripheral neuropathy, and nerve density in skin biopsies in 19 female patients with FD and 19 sex- and age-matched controls. They found that sensory nerve action potential amplitude and maximal sensory conduction velocity were not different, whereas there was a highly significant reduction in intraepidermal nerve fiber density; however, there was no correlation between pain and visual analog scale (VAS) score, QST, and intraepidermal nerve fiber density (Torvin Moller et al., 2009).

Further, van der Tol et al. (2016) assessed the diagnostic value of QST and IENFD testing in patients with an indeterminate FD diagnosis. Twenty-six patients were tested, 18 with nonclassical FD, 5 without FD, and 3 uncertain. The investigators found that "of the patients classified as nonclassical FD, 28% had ≥1 abnormal QST modalities, and 83% had an abnormal IENFD. From the patients without FD, 20% had ≥1 abnormal QST modality, and IENFD was abnormal in 25%" (van der Tol et al., 2016). Overall, the sensitivity was 28% and specificity was 80%.

von Cossel et al. (2021) studied the significance of the Fabry-related, non-classical variant p.D313Y in female patients. Nine females carrying the p.D313Y variant underwent intraepidermal nerve fiber density testing and results were compared to reference values. Compared to sex-matched reference values per decade, intraepidermal nerve fiber density was decreased in seven out of nine patients. Patients experienced acral paresthesia, neuropathic pain, and acute pain crises. The diagnosis of small fiber

neuropathy was made in seven out of nine females carrying the non-classical variant p.D313Y. The authors conclude that neuropathic pain and other symptoms related to autonomic nervous system dysfunction may be of clinical significance and warrant therapeutic intervention (von Cossel et al., 2021).

Parkinson Disease (PD)

Jeziorska et al. (2019) explored the relationship between nerve degeneration/regeneration and the clinical signs of Parkinson disease (PD). Twenty-three PD patients and 10 controls underwent IENF and clinical assessment. IENFD, total length (IETNFL), mean axonal length (MAL), and IETNFL/Area were all found to be reduced in PD patients. IENFD also correlated with disease duration and clinical measures of PD such as the Unified Parkinson's Disease Rating Scale, Part III. The authors concluded that "increased IENF degeneration and impaired regeneration correlates with somatic and autonomic symptoms and deficits in patients with PD" (Jeziorska et al., 2019).

Lim et al. (2021) studied the use of corneal confocal microscopy (CCM) to identify Parkinson's Disease (PD) patients with rapid motor progression. 64 patients with PD were assessed at baseline and at 12 month follow up for assessment on corneal nerve fiber density (CNFD), corneal nerve branch density (CNBD), corneal nerve fiber length (CNFL), corneal total branch density (CTBD), and corneal nerve fiber area. All four parameters were significantly lower in participants with PD compared with healthy control subjects. The mean difference between PD patients at baseline and control subjects were measured for CNFD (4.55 no./mm^2), CNBD (8.18 no./mm^2), CNFL (2.53 mm/mm^2), and CTBD (11.19 no./mm^2). The authors suggests that "CCM may be a useful marker of neurodegeneration to identify patients with PD with a more progressive and severe disease phenotype, termed "fast progressors" (Lim et al., 2021).

Charcot-Marie-Tooth Disease Type 1A (CMT1A)

Duchesne et al. (2018) investigated whether unmyelinated fibers are lost in Charcot-Marie-Tooth disease type 1A (CMT1A). Eighty CMT1A patients and 94 healthy controls provided skin biopsies from the distal leg, and the IENFD was calculated. The mean IENFD was found to be less in CMT1A patients compared to healthy controls (5.8 vs 9.57), and 48% of CMT1A patients had a reduction of IENFD below the "normal lower limit" of the fifth percentile of 4.8/mm. IENFD was also noted to decrease with age and to be higher in females than males. The authors suggested that small sensory nerve fibers were affected in CMT1A (Duchesne et al., 2018).

Ehlers-Danlos Syndrome (EDS)

Cazzato et al. (2016) investigated neuropathy in 20 adults with joint hypermobility syndrome/hypermobility Ehlers-Danlos syndrome (EDS), three patients with vascular EDS, and one patient with classic EDS. They found that all except one patient had neuropathic pain, but sural nerve conduction was normal in all patients. All patients showed decreased intraepidermal nerve fiber density consistent with small fiber neuropathy regardless of EDS type. The authors concluded that "small fiber neuropathy is a common feature of Ehlers-Danlos syndromes, and that skin biopsy could be considered an additional diagnostic tool to investigate pain manifestations in EDS" (Cazzato et al., 2016).

Friedreich's Ataxia (FRDA)

Indelicato et al. (2018) explored the association between Friedreich's ataxia (FRDA) and IENF. Seventeen patients with FRDA were enrolled. The mean IENF density was found to be lower in FRDA patients compared to healthy controls (5.77 ± 4.68 vs 9.33 ± 1.41 / mm). IENF was also found to be lower in early-onset FRDA patients compared to late-onset patients (early-onset median value: 1.7, late-onset

median value: 8.8). From there, a correlation between IENF density and shorter GAA repeat in FRDA patients was determined (r^2 = 0.573) (Indelicato et al., 2018).

Sarcoidosis

Gavrilova et al. (2021) studied the correlation of small fiber neuropathy and sarcoidosis. The study included 50 patients with pulmonary sarcoidosis and 25 healthy controls. A punch biopsy of the skin and staining with PGP 9.5 was performed. "A negative, statistically significant correlation between the intraepidermal nerve fiber density (IEND) and SFN-SL score was revealed." In Sarcoidosis patients, the median IEND in 1mm was 7.68. The authors conclude that small fiber neuropathy and sarcoidosis are correlated and "small fiber neuropathy might develop as a result of systemic immune-mediated inflammation" (Gavrilova et al., 2021).

Guidelines and Recommendations

American Academy of Neurology (AAN), American Association of Neuromuscular and Electrodiagnostic Medicine (AANEM) and the American Academy of Physical Medicine and Rehabilitation (AAPM&R)

A committee of the AAN, AANEM and AAPM&R published guidance on IENF density's use (England et al., 2009a):

- "Autonomic testing should be considered in the evaluation of patients with polyneuropathy to document autonomic nervous system dysfunction (Level B)."
- "Nerve biopsy is generally accepted as useful in the evaluation of certain neuropathies as in patients with suspected amyloid neuropathy, mononeuropathy multiplex due to vasculitis, or with atypical forms of chronic inflammatory demyelinating polyneuropathy (CIDP). However, the literature is insufficient to provide a recommendation regarding when a nerve biopsy may be useful in the evaluation of DSP (Level U)."
- "Skin biopsy is a validated technique for determining intraepidermal nerve fiber density and may be considered for the diagnosis of DSP, particularly SFSN (Level C). There is a need for additional prospective studies to define more exact guidelines for the evaluation of polyneuropathy."

The American Academy of Neurology reaffirmed these guidelines on January 22, 2022 (AAN, 2022).

American Association of Clinical Endocrinologists (AACE) and American College of Endocrinology (ACE)

The 2015 AACE and ACE review of the literature, by Garber et al. (2015), in development of a comprehensive diabetes management algorithm found that skin punch biopsy, a minimally invasive procedure, allows morphometric quantification of intraepidermal nerve fibers. The European Federation of the Neurological Societies and the Peripheral Nerve Society endorse intraepidermal nerve fiber quantification to confirm the clinical diagnosis of SFN with a strong recommendation (EFNS, 2010). Intraepidermal nerve fiber density inversely correlates with both cold and heat detection thresholds (Shun et al., 2004). Intraepidermal nerve fiber density is significantly reduced in symptomatic patients with normal findings from nerve conduction studies and those with metabolic syndrome, IGT, and IFG, suggesting early damage to small nerve fibers (Loseth et al., 2008; Quattrini et al., 2007). Intraepidermal nerve fiber density is also reduced in painful neuropathy compared with that observed in painless neuropathy (Sorensen et al., 2006). Diet and exercise intervention in IGT lead to increased intraepidermal nerve fiber density (Smith et al., 2006). These data suggest that intraepidermal nerve fiber loss is an early

feature of the metabolic syndrome, prediabetes, and established DM, and the loss progresses with increasing neuropathic severity. There may be nerve regeneration with treatment.

A consensus statement by the AACE and ACE on the Type 2 diabetes management algorithm was published in 2020. This statement was released in the form of an executive summary and does not mention skin punch biopsies or the quantification of intraepidermal nerve fibers (Garber et al., 2020).

In 2017, AACE (Vinik et al., 2017) published a position statement on nerve dysfunction that recommends:

- The presence of silent or overt autonomic neuropathy has dire consequences for the patient with diabetes, particularly if accompanied by peripheral neuropathy.
- All patients with type 2 diabetes should be assessed for both peripheral neuropathy at diagnosis and after 5 years, in type 1 diabetes at diagnosis and thereafter annually.
- Somatic neuropathy can be diagnosed by bedside testing with a 10-gram monofilament and a 128-Hz tuning fork for vibration perception and touch and prickling pain perception and ankle reflexes. This can be complemented by rapid and easily quantified sensory and sudomotor perception.

They found that: "It is a noninvasive objective test, takes a mere 2 minutes, has a sensitivity for diagnosis of neuropathy >75% and a specificity of 95%. These statistics have now been supported in studies by several authors amongst others and provide sensitive and specific diagnostic criteria for somatic neuropathy, which when combined with indices of HRV, provide better predictive value for CVD and mortality than traditional risk factors such as the tried and tested Framingham predictive index" (Vinik et al., 2017).

European Federation of Neurological Societies (EFNS) and Peripheral Nerve Society (PNS)

The EFNS/PNS published guidelines on the use of skin biopsy in the diagnosis of small fiber neuropathy which recommended that "Distal leg skin biopsy with quantification of the linear density of intraepidermal nerve fibers (IENF), using generally agreed upon counting rules, is a reliable and efficient technique to assess the diagnosis of SFN." EFNS added that "sweat gland innervation can be examined using an unbiased stereologic technique recently proposed. A reduced IENF density is associated with the risk of developing neuropathic pain, but it does not correlate with its intensity. Serial skin biopsies might be useful for detecting early changes of IENF density, which predict the progression of neuropathy, and to assess degeneration and regeneration of IENF. However, further studies are warranted to confirm the potential usefulness of skin biopsy with measurement of IENF density as an outcome measure in clinical practice and research. Skin biopsy has not so far been useful for identifying the etiology of SFN. Finally, we emphasize that 3-mm skin biopsy at the ankle is a safe procedure based on the experience of 10 laboratories reporting absence of serious side effects in approximately 35,000 biopsies and a mere 0.19% incidence of non-serious side effects in about 15 years of practice" (EFNS, 2010).

The EFNS also published guidance on assessment of neuropathic pain. In it, they recommend:

- "Skin biopsy should be performed in patients with painful/burning feet of unknown origin and clinical impression of small fibre dysfunction (grade B)."
- "In postherpetic neuralgia, skin innervation is reduced (grade B) and higher numbers of preserved fibres are associated with allodynia (grade B)."
- "IENFD shows only a weak negative correlation with the severity of pain and cannot be used to measure pain in individual patients (grade C)" (Cruccu et al., 2010).

American Diabetes Association (ADA)

In 2017 the ADA released a position statement on the early recognition and appropriate treatment of diabetic neuropathies which only mentions intraepidermal nerve fiber density as a measure of small fiber damage and repair in the context of clinical trials (Pop-Busui et al., 2017).

In the Standards of Medical Care in Diabetes, the ADA recommends that "All patients should be assessed for [diabetic peripheral neuropathy] starting at diagnosis of type 2 diabetes and 5 years after the diagnosis of type 1 diabetes and at least annually thereafter." (Grade B). Concerning the mode of assessment, they recommend, "Assessment for distal symmetric polyneuropathy should include a careful history and assessment of either temperature or pinprick sensation (small-fiber function) and vibration sensation using a 128-Hz tuning fork (for large-fiber function). All patients should have annual 10-g monofilament testing to identify feet at risk for ulceration and amputation (ADA, 2018, 2020, 2021, 2022, 2023)" (Grade B). They note the importance of diagnosis since "numerous treatment options exist for symptomatic diabetic neuropathy" (ADA, 2019).

International Expert Panel on Neuropathy in Fabry Disease

An international expert panel (Burlina et al., 2011) focused on early diagnosis of peripheral nervous system involvement in Fabry disease recommended: "Given the availability of an accurate diagnostic laboratory test, nerve or skin biopsies are not required for diagnosing Fabry disease, although skin biopsy can detect small fiber disease in yet asymptomatic patients and may be used to quantify loss of skin innervation" (Burlina et al., 2011).

Initiative on Methods, Measurement, and Pain Assessment in Clinical Trials (IMMPACT)

IMMPACT released guidelines on sensory testing, skin biopsy, and functional brain imaging as biomarkers in chronic pain clinical trials. Their guidance on skin biopsy is as follows:

- "Skin biopsy may be a useful tool to diagnose small fiber neuropathy (SFN) and may allow for earlier diagnosis of neuropathy and neuropathic pain conditions."
- "Although IENFD has promise as a diagnostic tool, it is important to recognize that in many of the data presented, IENFD was used to diagnose peripheral neuropathies that may or may not involve pain, rather than specifically to diagnose pain conditions themselves. In order to utilize IENFD as a diagnostic biomarker, additional research is needed that focuses specifically on the identification of pain conditions. Further research should also seek to validate the use of IENFD as a diagnostic tool for FM" (Smith et al., 2017).

Assessment Committee of the Neuropathic Pain Special Interest Group (NeuPSIG) of the International Association for the Study of Pain (IASP)

NeuPSIG released guidelines on neuropathic pain, with two recommendations relevant to skin biopsy. These are as follows:

- "Skin biopsy with appropriate histological processing and image analysis of the specimen should be performed in patients with clinical signs of small fiber dysfunction to determine intraepidermal nerve fiber density (level B)."
- "Measurement of intraepidermal nerve fiber density may be used in the follow up and to detect a treatment response in diabetic patients with small fiber neuropathy (level C)" (Haanpaa et al., 2011)."

Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
88313	Special stain including interpretation and report; Group II, all other (eg, iron, trichrome), except stain for microorganisms, stains for enzyme constituents, or immunocytochemistry and immunohistochemistry
88341	Immunohistochemistry or immunocytochemistry, per specimen; each additional single antibody stain procedure (List separately in addition to code for primary procedure)
88342	Immunohistochemistry or immunocytochemistry, per specimen; initial single antibody stain procedure
88344	Immunohistochemistry or immunocytochemistry, per specimen; each multiplex antibody stain procedure
88346	Immunofluorescence, per specimen; initial single antibody stain procedure
88350	Immunofluorescence, per specimen; each additional single antibody stain procedure (List separately in addition to code for primary procedure)
88356	Morphometric analysis; nerve

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

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Onychomycosis Testing

Policy Number: AHS – M2172 – Onychomycosis Testing	Prior Policy Name and Number, as applicable:
Initial Presentation Date: 03/10/2020 Revision Date: 03/06/2024	

Policy Description

Onychomycosis, also known as tinea unguium (Wollina et al., 2016), is a fungal infection of the nail typically caused by pathogenic fungal dermatophytes, such as *Trichophyton rubrum*, *Trichophyton mentagrophytes,* and *Epidermophyton floccossum;* onychomycosis may also be caused by yeasts, including *Candida parapsilosis* and *Candida guilliermondii*, or non-dermatophyte molds, including *Neoscytalidium dimidiatum, Onychocola canadensis,* the *Aspergillus* species, *Scopulariopsis* species, *Alternaria* species, *Acremonium* species, and *Fusarium* species (Ameen et al., 2014; Bongomin et al., 2018; Wollina et al., 2016). Terms such as male and female are used when necessary to refer to sex assigned at birth.

Related Policies

Policy Number	Policy Title
AHS-G2149	Pathogen Panel Testing
AHS-M2097	Identification Of Microorganisms Using Nucleic Acid Probes

Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) For individuals with onychomycosis, direct microscopic examination with potassium hydroxide, fungal culture of desquamated subungual material, or fungal stain of a nail clipping(s) **MEETS COVERAGE CRITERIA**.

2) For individuals with onychomycosis and for whom anti-fungal therapy has failed to resolve infection, nucleic acid amplification testing (NAAT) **MEETS COVERAGE CRITERIA.**

3) To screen for, diagnose, or confirm onychomycosis, NAAT (see Note 1) **DOES NOT MEET COVERAGE CRITERIA**.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness .

4) To screen for, diagnose, or confirm onychomycosis, attenuated total-reflectance fourier transform infrared (ATR-FTIR) spectroscopy **DOES NOT MEET COVERAGE CRITERIA**.

5) Testing for the presence of fungal-derived sterols (e.g., ergosterol) **DOES NOT MEET COVERAGE CRITERIA**.

NOTES:

Note 1: Nucleic acid testing (e.g., PCR, PCR-RFLP, and next-generation sequencing [NGS]) of the following microorganisms: *Candida* species, *Aspergillus* species, *Trichophyton rubrum, Trichophyton mentagrophytes, Epidermophyton floccossum, Neoscytalidium dimidiatum, Onychocola canadensis, Scopulariopsis* species, *Alternaria* species, *Acremonium* species, and *Fusarium* species (Ameen et al., 2014; Bongomin et al., 2018; Wollina et al., 2016).

Table of Terminology

Term	Definition
AAFP	American Academy of Family Physicians
AAP	American Academy of Pediatrics
ATR-FTIR	Attenuated total-reflectance fourier transform infrared
BAD	British Association of Dermatologists
CDC	Centers of Disease Control and Prevention
CLIA '88	Clinical Laboratory Improvement Amendments Of 1988
CMS	Centers For Medicare and Medicaid Services
CPS	Canadian Paediatric Society
DLSOM	Distolateral subungual onychomycosis
DNA	Deoxyribonucleic acid
FDA	Food and Drug Administration
GC	Gas chromatography
GC/MS	Gas chromatography–mass spectrometry
HIV	Human immunodeficiency virus
HPLC	High performance liquid chromatography
HPLC/MS	High performance liquid chromatography/mass spectrometry
ITS	Internal transcribed spacer

KOH	Potassium hydroxide
LC	Liquid chromatography
LC/MS	Liquid chromatography with tandem mass spectrometry
LDTs	Laboratory-developed tests
NAAT	Nucleic acid amplification testing
NDMs	Non-dermatophyte moulds
NGS	Next-generation sequencing
OSI	Onychomycosis severity index
PAS	Periodic acid-schiff
PCR	Polymerase chain reaction
PCR-RFLP	Polymerase chain reaction-restriction fragment length polymorphism
rDNA	Ribosomal DNA
RNA	Ribonucleic acid
SSI	*Streptomyces* subtilisin inhibitor
TDOM	Total dystrophic onychomycosis

Scientific Background

Onychomycosis is a fungal infection of the nail that causes approximately 50% of nail disease cases (Gupta et al., 2017) and is considered the most common nail disorder based on clinical statistics (Lipner & Scher, 2019). Onychomycosis infections can be obtained through several sources, including hotel carpets, bathtubs, saunas, pool decks, and public showers, and may be generated by dermatophytes, yeast, or mold. Data show that toenails are impacted 25 times more often than fingernails (Bongomin et al., 2018), and the first and fifth toe nail are more likely to be infected owing to the fact that footwear more frequently damages these nails (Ameen et al., 2014).

Dermatophytes are pathogenic fungi that can infect the skin, hair, and/or nails (Koo et al., 2019), and they are estimated to cause 90% of onychomycosis toenail cases and 50% of fingernail cases (Bodman & Krishnamurthy, 2022). These fungi attach to a surface such as an epithelial cell, extract nutrients, and grow as hyphae or filaments forming molds; this process allows the dermatophyte to seed several conditions, including onychomycosis (tinea unguium), athlete's foot (tinea pedis), and scalp ringworm (tinea capitis) (Achterman & White, 2013)**.** Wollina et al. (2016) suggest that an estimated 68% of onychomycosis cases are due to dermatophytes, 29% of cases due to yeasts, and 3% due to molds; further, mixed flora was identified in 5% to 15% of cases. Several types of dermatophytes may produce an onychomycosis infection, including *Trichophyton rubrum*, *Trichophyton mentagrophytes,* and *Epidermophyton floccossum* (Bodman & Krishnamurthy, 2022). In the United Kingdom, 85-90% of nail infections are due to dermatophytes (Ameen et al., 2014), while non-dermatophyte molds are estimated to cause between 2% to 25% of all onychomycosis cases (Bongomin et al., 2018). Non-dermatophyte mold onychomycosis causative agents include the *Aspergillus* species; incidence rates with this species vary between 1% to 35% of all cases and almost 71% in the elderly population (Bongomin et al., 2018).

A mature nail is comprised of the nail bed, nail plate, nail matrix, and nail fold (Wollina et al., 2016). Onychomycosis-causing pathogens live on the keratin of dead corneocytes and primarily infect the nail bed; after the nail bed thickens or becomes hyperkeratotic, the nail matrix is damaged (Bodman & Krishnamurthy, 2022). The nail plate may also be invaded during the infection, eventually becoming detached or warped, allowing the affliction to intensify (Bodman & Krishnamurthy, 2022). If a toenail case is not treated, the fungi, mold, or yeast could spread to the foot, causing tinea pedis in appropriate

conditions; infections may also spread to the hands or groin area (Ameen et al., 2014). If skin is externally disrupted, allowing bacteria entry into the body, the infection may also cause foot ulcers, cellulitis, osteomyelitis, and gangrene in diabetic patients (Ameen et al., 2014). While an official diagnosis requires lab results, typical visual cues for an onychomycosis infection include a jagged edge of the infected area of the nail "with spikes directed to the proximal fold, white-yellow longitudinal striae in the onycholytic nail plate, and colored parallel bands" (Abdallah et al., 2019). Subungual short spikes are also indicative of onychomycosis (Bodman & Krishnamurthy, 2022).

Several types of onychomycosis have been identified and include distolateral subungual (DLSOM), superficial white, proximal subungual, endonyx, and total dystrophic (TDOM) onychomycosis (Abdallah et al., 2019). Superficial white onychomycosis is rare, develops only in toenails, and occurs when the pathogens invade the nail through the nail plate; in proximal subungual onychomycosis, the infection occurs through the cuticle and typically develops in patients with a suppressed immune system (Wollina et al., 2016). Endonyx onychomycosis, which is caused by *T. soudanense,* occurs when the nail plate thickens; finally, the most advanced stage of onychomycosis is TDOM which may take up to 10 or 15 years to develop and can mature from any of the four main onychomycosis types mentioned above (Wollina et al., 2016).

The global prevalence of onychomycosis is estimated at 5.5% of the total population (Angulo-Rodríguez et al., 2021; Gupta et al., 2017). Ameen et al. (2014) estimate the onychomycosis prevalence in the United Kingdom at 3% of the adult population, while Wollina et al. (2016) estimate the prevalence in both the United States and Europe at 4.3% of the total population. Further, studies with a hospital-based population report an incidence at 8.9% (Wollina et al., 2016). Both lifestyle and general climate can impact infection rates.

As onychomycosis causes approximately 50% of nail disease cases, an estimated 15% of nail disorders can be contributed to metabolic conditions or inflammatory disorders, and five percent due to malignancies or pigment ailments (Wollina et al., 2016). Non-infectious nail diseases may include lichen ruber, yellow nail syndrome, psoriasis unguium, and tumors (Wollina et al., 2016). Onychomycosis may be stimulated by other nail disorders such as psoriasis (Ghannoum et al., 2018). When compared to nail psoriasis, onychomycosis infections tend to have more layers of parakeratosis, a greater amount of neutrophils and serous lakes, and a more blurred and/or irregular nail transition zone than psoriasis-based infections (Trevisan et al., 2019).

Several ailments or conditions increase the risk of an onychomycosis infection, including diabetes, obesity, old age, immunosuppression, smoking, human immunodeficiency virus (HIV) (Gupta et al., 2017), and cancer; further, patients who receive dialysis or who have previously received a transplant also experience a greater risk of developing an onychomycosis infection (Wollina et al., 2016). Diabetics are almost three times more likely to develop onychomycosis than non-diabetics; current data suggests that an estimated 34% of all diabetics have been diagnosed with the ailment (Ameen et al., 2014). Patients with HIV typically experience a more severe infection with all fingernails and toes infected due to a compromised immune system (Ameen et al., 2014). Onychomycosis is rare in pediatric populations, except in children with Down syndrome or immunodeficiencies (Solis-Arias & Garcia-Romero, 2017). Both men and older adults are more likely to develop onychomycosis compared to females or young adults (Ameen et al., 2014). These statistics could be contributed to the fact that older adults are more likely to exhibit reduced peripheral circulation, larger and potentially abnormal nail surfaces, difficulty grooming and maintaining efficient hygiene levels, and may have a greater chance of exposure to pathogenic fungi (Ameen et al., 2014). Athletes also experience onychomycosis infections at a greater incidence, with data suggesting that athletes are 2.5 times more likely to develop an infection than the

general population, with infections seven times more prevalent in toenails than fingernails (Daggett et al., 2019). This is likely due to the warm and moist environment in the shoe and sock, close quarters with other athletes, and/or trauma to the foot during sporting activities.

Proprietary Testing

An onychomycosis diagnosis should be given based on both clinical results and mycological lab results (Wollina et al., 2016). Several types of tests have been developed to diagnose onychomycosis. The current diagnostic gold standard includes direct microscopy with potassium hydroxide (KOH) and fungal culture, as these methods can identify the pathogenic species and fungal viability; additional tests include polymerase chain reaction (PCR) testing, fluorescent staining and periodic acid-Schiff (PAS) staining (Gupta et al., 2017; Rios-Yuil, 2017). It has been reported that KOH testing is only 60% sensitive and cannot identify the species, but it can differentiate between dermatophytes and saprophytes based on a positive result; "Currently, the most sensitive test (95%) is a pathologist interpreted nail clip biopsy that has been stained with periodic acid-Schiff (PAS) plus Grocott methenamine silver" (Bodman & Krishnamurthy, 2022). Mycologic culture may be used for suspected onychomycosis cases with negative KOH results if spores, hyphae, or other fungal structures were seen via microscopy; histologic evaluation of a nail clipping using PAS stain may assist in an onychomycosis diagnosis with more sensitive results than those given by mycologic culture (Arndt et al., 2016). An *Aspergillus* species causative agent may be suspected with a negative culture result but a positive KOH test (Bongomin et al., 2018). Fungal cultures must be interpreted by a mycologist and, while they are specific, they are only about 60% sensitive and take several weeks to grow (Bodman & Krishnamurthy, 2022). When utilized together, fungal culture and PCR can determine the source of the infection; the addition of PCR can improve species detection by 20% and will assist in differentiating between onychomycosis and nail dystrophy. PCR, when used with fungal culture, allows for a "much faster, highly sensitive, and very specific diagnosis" (Wollina et al., 2016). Multiplex qPCR assays have shown to be reliable for onychomycosis diagnostics with a shorter response time than traditional culture methods (Koo et al., 2019).

Many commercial tests are available.

For example, a multi-component test developed by Ipsum Diagnostics uses PCR to quickly identify the disease-causing agent in an onychomycosis infection alongside additional histology testing methods to provide same day results and evidence-based treatment options for both bacterial and fungal species (Ipsum Diagnostics, 2022).

SSI Diagnostica has developed a commercial Dermatophyte Real Time PCR Kit which allows for the diagnostic detection of dermatophytes in nail samples, particularly *T. rubrum* (SSI, 2023).

LabCorp has developed a fungus (mycology) culture test which analyzes a nail sample for an onychomycosis infection and delivers results in 24-42 days (LabCorp, 2023).

MicroGenDX offers a next-generation sequencing test to identify both bacterial and fungal species for nail infections. The test also provides a corresponding antibiotic list, based on antibiotic resistance genes detected. The test also prioritizes 16 items for 24-hour rapid results, which are as follows: "Methicillin resistance, Vancomycin resistance, Beta-lactam [resistance], Carbapenem [resistance], Macrolide [resistance], Aminoglycoside [resistance], Tetracycline [resistance], *Enterococcus faecalis Streptococcus agalactiae* (group B), *Streptococcus pyogenes* (group A), *Enterococcus faecium*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Candida albicans*, *Trichophyton rubrum*" (MicroGenDX, 2024).

Vikor Scientific has developed the Nail-ID™ test which uses advanced molecular PCR technology to deliver rapid results "through a value-based technology platform, ABXAssist™, which incorporates regional sensitivity and susceptibility patterns, medication costs, antibiotic spectrum of activity, and FDA guidance" (Vikor, 2020). The Nail-ID™ is able to deliver results in 24 hours after the sample is received, can detect polymicrobial infections simultaneously, and may identify as many as 49 antibiotic resistance genes to assist with treatment regimens (Vikor, 2020).

EuroImmun launched EuroArray Dermatomycosis, a PCR-based test that detects 56 fungi species causing skin, hair, and nail infections. This test detects 23 dermatophytes and six yeasts/molds in one reaction (EuroImmun, 2023).

Finally, BakoDx launched a Terbinafine resistance PCR test for Onychomycosis that detects 12 fungal mutations and terbinafine resistance in Trichophyton rubrum and Trichophyton mentagrophytes. This assay quickly detects resistance-associated mutations so that prescribing physicians can limit the use of ineffective medications and save patients time and costs. The assay has 99.9% specificity and 86% sensitivity (BakoDx, 2023).

Current onychomycosis treatments encompass antifungal medications (i.e. tavaborole and efinaconazole) and laser therapy; other treatments in the pipeline include iontophoresis and photodynamic therapy (Gupta et al., 2017). Dermatophyte infections may be treated with fluconazole, terbinafin, or itraconazole, while *Candida spp.* infections respond best to fluconazole (Wollina et al., 2016). Oral antifungal treatments are effective, but typically cause several unwanted side effects; on the other hand, topical antifungal treatments are less effective due to difficulties penetrating the nail but cause minimal side effects (Leung et al., 2020). If the nail matrix is involved, which can typically be identified by yellow streaks tarnishing the nail, both a systemic and topical antimycotic drug are recommended (Wollina et al., 2016). Treatments may occur over a period of months or years before an improvement is noticed; further, a toenail onychomycosis infection is reportedly more difficult to treat than a fingernail infection, and a recurrence rate is estimated between 5% to 50% (Bodman & Krishnamurthy, 2022). An article by Gupta et al. (2019) report that a relapse is likely to occur within the first 2.5 years after the infection has been cured; moreover, they state that to maximize cure rates, biofilms should be disrupted, drugs with more than one route of delivery should be utilized, and non-traditional treatments should be used in a timely manner if initial treatments are not efficient. Preventive strategies include retaining clean footwear, keeping toenails short and using topical antifungal agents.

Other fungal infections, such as dermatophytoma, may occur with onychomycosis infections, making these infections harder to treat; dermatophytoma can typically be identified "as a dense concentration of fungal hyphae within or under the nail plate and is generally white or yellow/brown in color, and linear (streaks) or round (patches) in shape" (Aly et al., 2018). A classification system has been developed to categorize the severity of an onychomycosis infection, termed the Onychomycosis Severity Index (OSI) (Carney et al., 2011). This score is determined by "multiplying the score for the area of involvement (range, 0-5) by the score for the proximity of disease to the matrix (range, 1-5). Ten points are added for the presence of a longitudinal streak or a patch (dermatophytoma) or for greater than 2 mm of subungual hyperkeratosis. Mild onychomycosis corresponds to a score of 1 through 5; moderate, 6 through 15; and severe, 16 through 35" (Carney et al., 2011).

Analytical Validity

Fungal fluorescent staining and internal transcribed spacer (ITS) ribosomal DNA (rDNA) PCR sequencing methods were compared to traditional direct microscopy with KOH detection methods for

onychomycosis diagnostics; data from a total of 204 patients was used (Bao et al., 2018). Fungal fluorescent staining was found to have a sensitivity of 97% and a specificity of 89%, while ITS rDNA PCR had a sensitivity of 78% and a specificity of 90%; the researchers concluded that the "Use of fluorescence enhanced the sensitivity of direct examination by 12% compared with KOH. PCR-based sequencing increased the sensitivity by 6% compared with culturing" (Bao et al., 2018).

Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) is a PCR technique that can be used to diagnose onychomycosis developed by Lubis et al. (2018); this method was compared against the fungal culture gold standard. Samples were collected from 35 patients; this PCR-RFLP method was found to have a specificity of 28.57% and a sensitivity of 85.71% (Lubis et al., 2018). While the sensitivity is high, a low specificity may suggest that this technique be used alongside the gold standard for onychomycosis testing to further improve sensitivity instead of replacing the traditional diagnostic method altogether.

Joyce et al. (2019) measured the effectiveness of quantitative PCR and next-generation sequencing instead of traditional, but expensive, KOH and culture techniques in diagnosing 8,816 "clinically suspicious" toenail samples; approximately 50% of the toenail samples were found to contain fungi and bacteria. The authors stated that these "Molecular methods were successful in efficiently quantifying microbial and mycologic presence in the nail. Contributions from dermatophytes were lower than expected, whereas the opposite was true for nondermatophyte molds" (Joyce et al., 2019).

Gustafson et al. (2019) used a real-time PCR assay on 425 clinical samples of suspected onychomycosis; results were compared to traditional KOH microscopy results. "Of 425 clinical samples suspected of onychomycosis analyzed by fungal culture and PCR, 219 samples were positive for both (52% agreement). Of the 206 discordant samples, 95% were resolved in favor of PCR by DNA sequencing" (Gustafson et al., 2019). These researchers also analyzed a larger data set of 2,452 samples. It was identified that histopathology has a positivity rate of 85%, PCR had a positivity rate of 73% and culture had a positivity rate of 54%; "PCR outperformed culture compared to histopathology for sensitivity (80% versus 49%), specificity (92% versus 79%), positive predictive value (94% versus 77%), and negative predictive value (76% versus 52%)" (Gustafson et al., 2019).

De Bruyne et al. (2019) used attenuated total-reflectance Fourier transform infrared (ATR-FTIR) spectroscopy as an alternative method to diagnose onychomycosis; spectral differences were used for dermatophytes (1692-1606 and 1044-1004 cm^{-1}) as well as for nondermatophytes and yeasts (973-937 cm^{-1}). An accuracy rating of 96.9% was given when identifying between uninfected nails, and nails infected with either dermatophytes, yeasts, or nondermatophytes; further, when discriminating between dermatophytes, yeasts, and nondermatophytes, classification rates were given of 91.0%, 98.6% and 97.7% respectively (De Bruyne et al., 2019).

Liquid chromatography-tandem mass spectrometry has been used by Ho et al. (2019) to identify ergosterol, a sterol that most fungi cannot survive without, as a new diagnostic tool for fungal infected nails. Samples from 20 participants were collected and analyzed, which is a relatively small sample size. However, the researchers determined that this mass spectrometry diagnostic method "seemed to be better at detecting combinations of nail conditions" than current techniques, but further studies need to be completed to determine the sensitivity and specificity of this method (Ho et al., 2019).

Mourad et al. (2019) compared Chicago sky blue staining and Calcofluor white staining to traditional KOH wet mount and culture techniques; samples from 50 patients with dermatophytosis of the hair or nail were used. Both Chicago sky blue staining and Calcofluor white staining of the hair and nail were

found to be more specific and sensitive for the diagnosis of fungal infections when compared to traditional diagnostic methods because the KOH wet mount technique is reportedly a "simple, rapid, and inexpensive test but lacks color contrast and gave more false positive (artifacts) and false-negative results as compared to these new stain methods" (Mourad et al., 2019).

Caldwell et al. (2020) compared commercial multiplex PCR versus Periodic Acid–Schiff (PAF) testing for the diagnosis of Onychomycosis. A total of 209 Onychomycosis patients were recruited for the study and two toenail samples from each patient were sent for PCR and PAS testing. Of the 203 patients, "109 (53.7%) tested positive with PAS, 77 (37.9%) tested positive with PCR. Forty-one patients tested positive with PAS but negative with PCR, and nine tested positive with PCR but negative with PAS." The authors conclude that the clinical practice of PAS biopsy staining should continue for confirmation of a fungal toenail infection before treatment. PCR test may be added optionally as it allows for species identification (Caldwell et al., 2020).

Clinical Utility and Validity

The frequency of onychomycosis infections was measured in patients with psoriasis compared to controls by Romaszkiewicz et al. (2018); data from a total of 2527 patients was used, with 2325 patients presenting with nail abnormalities and onychomycosis suspicion with no previous history of psoriasis, 102 psoriatic patients with onychomycosis suspicion, and 100 controls. The researchers used direct microscopy and culture to identify fungal infections, and found that "The prevalence of onychomycosis did not differ significantly between psoriatic patients and non-psoriatic patients with nail alterations" (Romaszkiewicz et al., 2018). However, it was identified that the characteristics of the fungi isolated from the patients "differed significantly between psoriatic and non-psoriatic patients," which is important to note regarding treatment regimens (Romaszkiewicz et al., 2018). Another study, completed by Gallo et al. (2019), also measured onychomycosis prevalence between psoriatic and non-psoriatic patients; similar results were found. This study analyzed data from a total of 9281 patients and found similar infection rates between psoriatic and non-psoriatic groups; however, once again, the "spectrum of fungal species isolated was different," with patients in the non-psoriatic group more likely to be infected with yeasts than patients in the psoriatic group (Gallo et al., 2019).

A meta-analysis was completed by Velasquez-Agudelo and Cardona-Arias (2017) to determine the utility, validity and performance of culture, nail clippings with PAS staining, and KOH testing for onychomycosis diagnostic purposes; this meta-analysis search utilized "5 databases and 21 search strategies." Results showed that "The diagnostic tests evaluated in this meta-analysis independently showed acceptable validity, performance, and efficiency, with nail clipping with PAS staining outperforming the other two tests" (Velasquez-Agudelo & Cardona-Arias, 2017). Another study by Gupta et al. (2018) measured several types of onychomycosis confirmatory testing methods such as KOH, culture, and PAS. It was determined that PAS was once again "the most sensitive confirmatory test and KOH the least expensive"; incorrect diagnoses made without confirmatory tests led to the unnecessary spending of several hundred Canadian dollars, suggesting that confirmatory lab diagnostics are preferred before treatment (Gupta et al., 2018).

Martinez-Herrera et al. (2015) measured the number of onychomycosis cases due to opportunistic molds; this retrospective study analyzed data from 4220 onychomycosis cases and found that only 32 cases (0.76%) were caused by opportunistic molds. This study also found that the age group most affected was between 41 and 65 years old and that females were affected slightly more than males at 65.6% (Martinez-Herrera et al., 2015). Further, the authors also reported that "The most frequent isolated etiological agents were: *Aspergillus sp*. and *Scopulariopsis brevicaulis*" (Martinez-Herrera et al., 2015).

Haghani et al. (2019) examined the species distribution of "causative agents" of onychomycosis. A total of 257 patients contributed samples, and the agents in these samples were identified through PCR. Onychomycosis was identified in 180 cases, and "51.1% of these cases were caused by non-dermatophyte moulds (NDMs), 35% by yeast and 10.6% by dermatophytes." The authors also found that novel triazoles and imidazoles such as "efinaconazole, luliconazole and lanoconazole" showed "potent" activity compared to other antifungal agents. The authors concluded that "that obtained data will be useful to improve the knowledge of researchers, clinicians and dermatologists about onychomycosis distribution, species diversity and adoption of appropriate treatment" (Haghani et al., 2019).

Trave et al. (2021) studied the clinical utility of the EuroArray dermatomycosis kit, a PCR-based microarray to detect species involved in skin and nail infections. The researchers identified 100 patients suspected of onychomycosis who were evaluated based on three diagnostic methods: KOH preparation, culture, and EuroArray. Onychomycosis was diagnosed in 47 of 100 patients who were positive on at least one of three diagnostic tests and in 49 of 100 patients who were PCR-positive. Combining microscopy and PCR had better sensitivity than fungal culture, microscopy, and PCR alone. Culture rather than PCR resulted in more frequently positive results in molds, while dermatophytes were more frequently positive in both culture and PCR. *Trichophyton interdigitale* was the most frequent pathogen. The authors conclude that the EUROArray increased the sensitivity of microscopy and yields more rapid results than culture (Trave et al., 2021).

Guidelines and Recommendations

Centers of Disease Control and Prevention (CDC)

The CDC remarks that an onychomycosis infection may be diagnosed through visual inspection, questioning the patient on their symptoms, or a fungal culture. The CDC states "to confirm the diagnosis, the healthcare provider might collect a nail clipping to look at under a microscope or to send to a laboratory for testing". The following types of laboratory tests can confirm the diagnosis of onychomycosis:

- "Microscopy: Potassium hydroxide (KOH) stain can be performed in the office setting, but the accuracy of the test depends on clinician experience and technique. Nail clippings or scrapings are placed in a drop of KOH and examined under a microscope for the presence of fungal elements.
- Histopathologic examination with a periodic acid-Schiff (PAS) stain: Examination of nail clippings with a PAS stain can confirm the diagnosis of a fungal nail infection.
- Culture: Fungal culture can be used to identify the infecting organism, but the fungi may take several weeks to grow.
- Molecular: Molecular testing, including polymerase chain reaction (PCR) testing, may be used to diagnose fungal nail infections" (CDC, 2022).

The CDC also notes that the term "onychomycosis" is the technical term for a "fungal nail infection" (CDC, 2022).

American Academy of Pediatrics

Within the AAP's Red Book, recommendations include the following concerning diagnostic testing for onychomycosis: "Fungal infection of the nail (tinea unguium or onychomycosis) can be verified by direct

microscopic examination with potassium hydroxide, fungal culture of desquamated subungual material, or fungal stain of a nail clippings fixed in formalin" (AAP, 2018b).

The AAP also notes that confirmatory diagnostic tests are similar to those for tinea corporis. According to the AAP Red Book, fungal culture to diagnose tinea corporis can be used, but that "polymerase chain reaction and periodic acid-Schiff stain evaluation of specimens are available but are expensive and generally are not necessary" (AAP, 2018a).

British Association of Dermatologists (BAD)

The BAD have published guidelines for the management of onychomycosis stating that "The clinical characteristics of dystrophic nails must alert the clinician to the possibility of onychomycosis. Laboratory confirmation of a clinical diagnosis of tinea unguium should be obtained before starting treatment. This is important for several reasons: to eliminate nonfungal dermatological conditions from the diagnosis; to detect mixed infections; and to diagnose patients with less responsive forms of onychomycosis, such as toenail infections due to *T. rubrum*. Good nail specimens are difficult to obtain but are crucial for maximizing laboratory diagnosis. Material should be taken from any discoloured, dystrophic, or brittle parts of the nail" (Ameen et al., 2014).

Further, the BAD also stated that "Traditionally, laboratory detection and identification of dermatophytes consists of culture and microscopy, which yields results within approximately two to six weeks. Calcofluor white is exceedingly useful for direct microscopic examination of nail specimens, as the fungal elements are seen much more easily than with potassium hydroxide, thereby increasing sensitivity" (Ameen et al., 2014).

More recent molecular genetic tools were also highlighted as a newer diagnostic technique for the detection of dermatophytes. Regarding PCR testing, the BAD has stated that "Real-time polymerase chain reaction (PCR) assays have been developed, which simultaneously detect and identify the most prevalent dermatophytes directly in nail, skin and hair samples and have a turnaround time of < two days. It appears that real-time PCR significantly increased the detection rate of dermatophytes compared with culture. However, PCR may detect nonpathogenic or dead fungus, which could limit its use in identifying the true pathogen. Restriction fragment length polymorphism analysis, which identifies fungal ribosomal DNA, is very helpful for defining whether the disease is caused by repeat infection or another fungal strain when there is a lack of response to treatment. However, this technique has not been implemented into routine clinical practice" (Ameen et al., 2014).

Finally, the BAD also stated that "histopathological analysis using periodic acid–Schiff staining is more sensitive than direct microscopy or culture. However, this technique is not currently available in the majority of dermatology clinics or mycology laboratories. Other diagnostic techniques under investigation include flow cytometry and confocal and scanning electron microscopy" (Ameen et al., 2014).

Canadian Paediatric Society (CPS)

The CPS notes that treatment effectiveness will differ depending on the type of fungal or mold infection, and therefore highlights the importance of sending nail clippings for culture to "allow differentiation between dermatophyte and non-dermatophytic fungal nail infections." The CPS also remarks that "Terbinafine has excellent action against dermatophytes, but is less effective for *Candida* onychomycosis, and these cases are best treated with azoles" (Bortolussi & Martin, 2007). Reaffirmed in 2019.

The American Academy of Family Physicians (AAFP)

The AAFP published guidelines in 2013 regarding current trends in the diagnosis and treatment of onychomycosis. These guidelines suggested C evidence ratings for the following statements:

"Periodic acid–Schiff staining should be ordered to confirm infection in patients with suspected onychomycosis."

When preparing a nail specimen to test for onychomycosis, the nail should be cleaned with 70% isopropyl alcohol, then samples of the subungual debris and eight to 10 nail clippings should be obtained" (Westerberg & Voyack, 2013).

The AAFP also stated that an "Accurate diagnosis is crucial for successful treatment and requires identification of physical changes and positive laboratory analysis" (Westerberg & Voyack, 2013). Further, a diagnosis flowchart was given and states that if a nail is discolored or gives reason to suspect onychomycosis, nail clippings should be obtained and looked at under a microscope; if the microscopic viewing suggests a positive onychomycosis diagnosis, treatment should begin to identify the organism (treatment includes culture and/or histologic evaluations with periodic acid-Schiff staining) (Westerberg & Voyack, 2013).

In 2014, Ely et al. (2014) gave a C evidence rating when examining both "Tinea corporis, tinea cruris, and tinea pedis can often be diagnosed based on appearance, but a potassium hydroxide preparation or culture should be performed when the appearance is atypical" and "The diagnosis of onychomycosis should generally be confirmed with a test such as potassium hydroxide preparation, culture, or periodic acid–Schiff stain before initiating treatment."

The Journal of Drugs in Dermatology (JDD)

The Journal of Drugs in Dermatology released guidelines for diagnosis and treatment of toenail Onychomycosis in the US. For diagnosis and testing, JDD recommends that: confirmatory laboratory testing should be performed using one or more of the following: microscopic examination (eg, potassium hydroxide [KOH], periodic acid-Schiff test [PAS]), or fungal culture. While polymerase chain reaction (PCR) techniques were considered useful for confirming diagnosis, they were deemed not cost effective enough for general use" (Lipner et al., 2021).

Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, please visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). As an LDT,

the U. S. Food and Drug Administration has not approved or cleared this test; however, FDA clearance or approval is not currently required for clinical use.

Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
82542	Column chromatography, includes mass spectrometry, if performed (eg, HPLC, LC, LC/MS, LC/MS-MS, GC, GC/MS-MS, GC/MS, HPLC/MS), non-drug analyte(s) not elsewhere specified, qualitative or quantitative, each specimen
87101	Culture, fungi (mold or yeast) isolation, with presumptive identification of isolates; skin, hair, or nail
87149	Culture, typing; identification by nucleic acid (DNA or RNA) probe, direct probe technique, per culture or isolate, each organism probed
87150	Culture, typing; identification by nucleic acid (DNA or RNA) probe, amplified probe technique, per culture or isolate, each organism probed
87153	Culture, typing; identification by nucleic acid sequencing method, each isolate (eg, sequencing of the 16S rRNA gene)
87205	Smear, primary source with interpretation; Gram or Giemsa stain for bacteria, fungi, or cell types
87206	Smear, primary source with interpretation; fluorescent and/or acid fast stain for bacteria, fungi, parasites, viruses or cell types
87220	Tissue examination by KOH slide of samples from skin, hair, or nails for fungi or ectoparasite ova or mites (eg, scabies)
87480	Infectious agent detection by nucleic acid (DNA or RNA); Candida species, direct probe technique
87481	Infectious agent detection by nucleic acid (DNA or RNA); Candida species, amplified probe technique
87482	Infectious agent detection by nucleic acid (DNA or RNA); Candida species, quantification
87798	Infectious agent detection by nucleic acid (DNA or RNA), not otherwise specified; amplified probe technique, each organism
87800	Infectious agent detection by nucleic acid (DNA or RNA), multiple organisms; direct probe(s) technique
87801	Infectious agent detection by nucleic acid (DNA or RNA), multiple organisms; amplified probe(s) technique
88312	Special stain including interpretation and report; Group I for microorganisms (eg, acid fast, methenamine silver)
88749	Unlisted in vivo (eg, transcutaneous) laboratory service

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

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Revision History

Revision Date	Summary of Changes
03/06/2024	Reviewed and Updated: Updated background, guidelines, and evidence-based scientific references. Literature review necessitated the following changes in coverage criteria: Added new CC2 to allow for NAAT testing in situations where treatment has been ineffective and anti-fungal resistance is suspected. CC2 reads: "2) For individuals with onychomycosis and for whom anti-fungal therapy has failed to resolve infection, nucleic acid amplification testing (NAAT) MEETS COVERAGE CRITERIA." Inclusion of defining the NAAT initialism in CC2 results changing "nucleic acid testing" to "NAAT" in CC3.

03/01/2023	Reviewed and Updated: Updated the background, guidelines and recommendations, and evidence-based scientific references. Literature review did not necessitate any modifications to coverage criteria. The following edits were made for clarity: All CC edited for clarity and consistency.
03/09/2022	Annual review: Updated background, guidelines and recommendations, and evidence-based scientific references. Literature review did not necessitate any modification to coverage criteria.
03/03/2021	Annual review: Updated background, guidelines and recommendations, and evidence-based scientific references. Literature review did not necessitate any modification to coverage criteria.
03/10/2020	Initial presentation

Oral Cancer Screening and Testing

Policy Number: AHS – G2113 – Oral Cancer Screening and Testing	Prior Policy Name and Number, as applicable: AHS-G2113- Oral Screening Lesion Identification Systems and Genetic Screening
Initial Presentation Date: 06/16/2015 Effective Date: February 1, 2025	

Policy Description

Oral cancer is defined as cancer occurring in the oral cavity between the vermilion border of the lips and the junction of the hard and soft palates or the posterior one third of the tongue. Squamous cell carcinoma is the most common type of oral cancer (Gross et al., 2024).

Related Policies

Policy Number	Policy Title
	N/A

Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) To establish HPV tumor status for individuals with oropharyngeal squamous cell carcinoma, testing for high-risk HPV with either mRNA expression testing for HPV E6/E7 or immunohistochemistry for p16 expression **MEETS COVERAGE CRITERIA**.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

2) To screen, detect, or diagnose oral cancer, the following testing **DOES NOT MEET COVERAGE CRITERIA:**
   a) Salivary biomarker testing (e.g., peptides/proteins, nucleic acids, metabolites).
   b) Genotyping of HPV (e.g., OraRisk® HPV).
   c) Gene expression profiling.
   d) Panels that incorporate genetic risk factors with nongenetic biomarkers (e.g., mRNA CancerDetect™).

Table of Terminology

Term	Definition
8-OHdG	8-hydroxy-2′-deoxyguanosine
ACS	American Cancer Society
ADA	American Dental Association
AF	Auto-fluorescence
AHSG	Alpha-2-HS-glycoprotein
ASCO	American Society of Clinical Oncology
AUC	Area under curve
AZGP1	Zinc-alpha-2-glycoprotein
BPIFB2	Bactericidal/permeability-increasing protein fold containing family B member 2
CAP	College of American Pathologists
CD59	Cluster of differentiation 59
CDC	Centers for Disease Control and Prevention
CL	Chemiluminescence
CLIA '88	Clinical Laboratory Improvement Amendments of 1988
CMS	Centers for Medicare and Medicaid
COE	Conventional oral examination
CPT	Current procedural terminology
DNA	Deoxyribonucleic acid
DUSP1	*Dual specificity phosphatase 1*
EBER	Epstein-Barr-encoded ribonucleic acid
EBV	Epstein-Barr virus
EHNS	European Head and Neck Society
ESMO	European Society for Medical Oncology
ESTRO	European Society for Radiotherapy and Oncology
H3F3A	*H3 histone, family 3A*
HNSCC	Head and neck squamous cell carcinoma
HPV	Human papillomavirus

HR	High-risk
HR-HPV	High-risk human papillomavirus infection
IHC	Immunohistochemistry
IL-8	*Interleukin-8*
IL-1B	*Interleukin-1B*
KLK1	Kallikrein 1
KRT6C	Keratin 6C
LACRT	Lacritin
LBDS	Light-based detection systems
LC-MS	Light chromatography-mass spectrometry
LDTs	Laboratory-developed tests
LED	Light emitting diodes
M2BP	Mac-2 binding protein
MDA	Malondialdehyde
MED15	*Mediator complex subunit 15*
miRNA	Micro ribonucleic acid
mRNA	Messenger ribonucleic acid
MRP14	Migration inhibitory factor-related protein 14
MSP	Methylation-specific polymerase chain reaction
NCCN	National Comprehensive Cancer Network
OAZ1	*Ornithine decarboxylase antizyme 1*
OC	Oral cancer
OPC	Oropharyngeal cancer
OPMD	Oral potentially malignant disorders
OSCC	Oral squamous cell carcinoma
PCQAP	*Mediator complex subunit 15*
PMD	Potentially malignant disorder
RASSF1A	*Ras association domain family 1 isoform A (gene)*
RASSF1α	Ras association domain family 1 isoform A (protein)
RNA	Ribonucleic acid
S100P	*S100 Calcium Binding Protein P*
SAT	*Spermidine/spermine N1-acetyltransferase*
SCC	Squamous cell carcinoma
SCCUP	Squamous cell carcinoma of unknown primary
TIMP3	*TIMP metallopeptidase inhibitor 3*
USPSTF	United States Preventive Services Task Force

Scientific Background

The American Cancer Society (ACS) estimates the 2019 incidence of oral cancer to be 53,000 cases with approximately 10,860 deaths (Siegel et al., 2019). The American Cancer Society estimates that in the United States in 2024, approximately 58,450 people will be diagnosed with oral cavity and

oropharyngeal cancers and approximately 12,230 people will die from these cancers (ACS, 2024). Oral squamous cell carcinoma (OSCC) is the most common form of oral cavity cancer, which constitutes 94.08% of all epithelial tumors and 80.05% of all oral cancers (Dhanuthai et al., 2018; Scully & Porter, 2000). Many cases are preceded by a potentially malignant disorder (PMD), which is a heterogeneous group of conditions including erythroplakia, non-homogeneous leukoplakia, erosive lichen planus, oral submucous fibrosis and actinic keratosis (Warnakulasuriya et al., 2007). The early detection and excision of PMD can prevent malignant transformation (Brocklehurst et al., 2013; van der Waal, 2009; Warnakulasuriya et al., 2007)

Human papillomavirus (HPV) is a common sexually transmitted infection that may lead to the development of warts or cancer in various parts of the body including the back of the throat, tonsils, and base of the tongue. This type of cancer is known as oropharyngeal cancer. HPV is also a major contributor to the development of head and neck squamous cell carcinoma (HNSCC), which can develop in the mouth, nose, and throat (Borsetto et al., 2018). According to the CDC (2024), there is no test to determine an individual's HPV status, and "there is no approved HPV test to find HPV in the mouth or throat."

Diagnosing and treating dermatologic lesions of the mouth and gums is challenging for most clinicians because of the wide variety of disease processes that can present with similar appearing lesions and the fact that most clinicians receive inadequate training in mouth diseases (Lodi, 2024). Several index tests have been proposed as adjuncts to a conventional oral examination (COE) to improve diagnostic test accuracy (Fedele, 2009; Lingen et al., 2008; Patton et al., 2008; Rethman et al., 2010; Seoane Leston & Diz Dios, 2010). These tests include vital staining, brush cytology, and blood or saliva analysis. These screening tests are not only used for diagnostic purposes but can also be utilized as a tool to measure any changes that may be signs of future disease development (Speight et al., 2017).

Additionally, blood or saliva can be tested for biomarkers for cancer. The tests are non-invasive but have low standardization and are not widely used in clinical practice (Macey et al., 2015). Nonetheless, saliva has been identified as an ideal diagnostic medium for the early detection of HNSCC activity because it is close to the tumor site and is an easy sample to obtain (Lim et al., 2016). Macey et al. (2015) concluded that none of the adjunctive biomarker tests can be recommended as a replacement for the currently used standard of COE followed by a scalpel biopsy and histological assessment. However, the NCCN has stated that that "Expression of p16 as detected by IHC [immunohistochemistry] is a widely available surrogate biomarker that has a very good agreement with HPV status as determined by the gold standard of HPV E6/E7 mRNA expression" (NCCN, 2024). The protein known as p16 slows cell division, therefore acting as a tumor suppressor. Researchers have identified *p16^{INK4a}*, *RASSF1A, TIMP3*, and *PCQAP/MED15* as tumor suppressor genes that exhibited "excellent diagnostic accuracy in the early detection of OC [oral cancer] at 91.7% sensitivity and 92.3% specificity and of OPC [oropharyngeal cancer] at 99.8% sensitivity and 92.1% specificity from healthy controls" (Liyanage et al., 2019). A review by Kaur et al. (2018) that researched salivary biomarkers for oral cancer and pre-cancer screening have identified a plethora of salivary biomarkers which showed an improvement in oral cancer diagnoses including mRNAs, salivary transcriptomes (*IL-8, IL-1B, DUSP1, H3F3A, OAZ1, S100P*, and *SAT* were highly specific (91%) and sensitive (91%) for oral cancer detection), and salivary biomarkers (M2BP, profilin, CD59, MRP14, and catalase had a sensitivity of 83% and a specificity of 90% for oral cancer detection)" (Kaur et al., 2018).

The OraRisk® HPV by OralDNA Labs is a salivary diagnostic test that analyzes the molecular genotypes of HPV. The test can identify a total of 51 types of oral HPV including high-risk, low-risk and unknown-risk genotypes. High Risk Genotypes: 16, 18, 26, 30, 31, 33, 34, 35, 39, 45, 51, 52, 53, 56, 58, 59, 64, 66, 67,

68, 69, 70, 73, 82. Low Risk Genotypes: 2a, 6, 11, 32, 40, 42, 43, 44, 54, 55, 57, 61, 62, 71, 72, 74, 77, 81, 83, 84, 89. Unknown Risk Genotypes: 41, 49, 60, 75, 76, 80, 85 (OralDNA, 2023).

Clinical Utility and Validity

Nagi et al. (2016) conducted a systematic review to evaluate the effectiveness of adjunctive devices that utilize the principles of chemiluminescence and tissue autofluorescence in the detection of oral squamous cell carcinoma (OSCC) and oral potentially malignant disorders (OPMD). Twenty primary studies published satisfied the criteria for selection. Ten used chemiluminescence and 10 used tissue autofluorescence. ViziLite was used for evaluation of chemiluminescence, and it was evaluated at a sensitivity of 0.771 to 1.00 and specificity of 0.00 to 0.278. Tissue autofluorescence was evaluated with VELscope. This technique was evaluated at a sensitivity of 0.22-1.00 and specificity of 0.16 to 1.00. The authors concluded that more clinical trials in the future should be conducted to establish optical imaging as an efficacious adjunct tool in early diagnosis of OSCC and OPMD (Nagi et al., 2016).

Shaw et al. (2022) conducted a systematic review to compare the existing evidence on diagnostic accuracy of salivary biomarkers with their estimation method in detecting early oral squamous cell carcinoma. Salivary biomarkers provide promising complementary alternative diagnostic adjunct for its simple non- invasive collection and technique and to screen large population. "18 studies were included for qualitative synthesis, and out of that 13 for meta-analysis. Sensitivity and specificity were calculated with AUC. For mRNA it was 91% and 90% with 0.96 AUC, miRNA had 91% and 91% with 0.95 AUC for PCR. IL-1B had 46% and 60% with 0.61 AUC, S100p had 45% and 90% with 0.57 AUC for ELISA. IL-8 had 54% and 74% for ELISA and 89% and 90% for PCR with 0.79 AUC and DUSP1 had 32% and 87% for ELISA and 76% and 83% for PCR with 0.83 AUC respectively. Early detection of OSCC was best achieved by screening for salivary mRNA and miRNA estimated by PCR" (Shaw et al., 2022).

Lingen et al. (2017); Lingen et al. (2008) performed a meta-analysis of the screening adjuncts for oral cancer. The authors evaluated cytologic adjuncts as well as vital staining, tissue reflectance, autofluorescence, and salivary biomarkers. The vital staining cohort included 15 studies with 1453 lesions and was evaluated at a 0.87 sensitivity and 0.71 specificity. The tissue reflectance cohort (5 studies, 390 lesions) was assessed at a 0.72 sensitivity and 0.31 specificity. The autofluorescence segment (7 studies, 616 lesions) was computed at a 0.90 sensitivity and a 0.72 specificity. The authors stated, most biomarkers showed a wide range of diagnostic test accuracy results, "with sensitivity ranging from 0.5 to 0.9 and specificity ranging from 0.63 to 0.9." Finally, cytology (15 studies, 2148 lesions) was assessed at a 0.92 sensitivity and 0.94 specificity. The authors concluded that cytology appeared to be most accurate adjunct (Lingen et al., 2017).

Another systematic review was completed that focused on the use of oral brush cytology for the early detection of oral cancer and OPMDs (Alsarraf et al., 2018). Thirty-six of the 343 abstracts and articles identified met the inclusion criteria, with publication dates ranging from 1994 to 2017. These articles led to the inclusion of 4302 total samples from OPMDs, oral squamous cell carcinoma, and healthy controls. The results were somewhat troubling. "Findings from this study indicate that meaningful evidence-based recommendations for the implementation of a minimally invasive technique to be utilized as an adjunctive tool for screening and early detection of oral cancer and OPMDs are complicated from the reported studies in the literature" (Alsarraf et al., 2018).

Kaur et al. (2018) completed a review which focused on salivary biomarkers for oral cancer and pre-cancer screening. A total of 270 articles published between 1995 and 2017 were identified for this review. The authors note that biomarkers may be arranged into four categories: normal health (*IL-8, IL-*

1β, etc.), general health (glycolytic enzyme lactate dehydrogenase, etc.), specific (*S100P* mRNA for cancer), and non-specific salivary (8-OHdG and MDA biomarkers of oral cancer and pre-cancer) (Kaur et al., 2018). Results from this study led to the conclusion that "Biomarkers such as methylation markers, IL-8, actin, myosin, and miRNAs are very speculative and remain without sufficient scientific evidence when it comes to oral cancer and pre-cancer detection using body fluids. Salivary peptides such as protein 14, Mac-2 binding protein, profilin 1, CD59, defensin-1, catalase proteins, etc. with sensitivity approximating 90% and specificity 80% for oral cancer diagnosis have been described"; "Furthermore, five salivary metabolites such as valine, lactic acid, and phenylalanine in combination yielded satisfactory accuracy (0.89), sensitivity (94.6%), and specificity (84.4%) in distinguishing oral cancer from controls or oral pre-cancer, respectively" (Kaur et al., 2018). Based on the results in this large group of studies, the researchers state that the "Combination approach of salivary biomarkers could be used as [a] screening tool to improve early detection and diagnostic precision of oral pre-cancer and cancer" (Kaur et al., 2018). The findings of this extensive review highlight that it is important for researchers to mitigate the current challenges involved with the use of salivary biomarkers for oral cancer and pre-cancer screening as this technique has the potential to improve early detection and diagnostic methods.

Using "targeted proteomics, identified initially by relative quantification of salivary proteins on LC-MS [light chromatography-mass spectrometry]," Jain et al. (2021) identified a potential salivary biomarker panel having been motivated by the high prevalence, incidence, and mortality of oral cancer/oral squamous cell carcinoma among Indians. In a case-control cohort study, "Out of the twelve proteins validated, two proteins AHSG and KRT6C were significantly upregulated and four proteins, AZGP1, KLK1, BPIFB2 and LACRT were found to be significantly downregulated," but when accounting for tobacco consumption habits, "AHSG and AZGP1 were dysregulated in cases compared to controls irrespective of their tobacco consumption habits. While KRT6C, KLK1 and BPIFB2 were significantly dysregulated only in the cases having tobacco consumption habits." AZGP1 is important in insulin sensitivity and the cell cycle; KLK1 is a serine protease involved in "remodelling of the extracellular matrix, cellular proliferation and differentiation, angiogenesis, and apoptosis;" BPIFB2 is a lipid transfer/lipopolysaccharide binding protein that is not well understood in cancer; KRT6C is a type II keratin subtype and is expressed in "filiform papillae of the tongue, stratified epithelial lining of the oesophagus, and oral mucosa and in glandular epithelia;" and AHSG is involved in "multiorgan expression during embryogenesis," but is mostly in the liver and some osteoblasts in adults. In their risk prediction model, AZGP1, AHSG, and KRT6C had sensitivities of 82.4%, 78%, and 73.5%, respectively for all stages of OSCC, and 87.9%, 87.5%, and 73.5%, respectively for late stage OSCC (Jain et al., 2021).

Lim et al. (2016) competed a study to determine the diagnostic ability of four HNSCC biomarkers (RASSF1α, $p16^{INK4a}$, TIMP3, PCQAP/MED15) isolated from saliva. The DNA methylation status of these biomarkers was measured via methylation-specific PCR (MSP). Data from a total of 88 HNSCC patients and 122 healthy controls was analyzed. The authors found that a "salivary DNA tumour-suppressor methylation gene panel has the potential to detect early-stage tumours in HPV-negative HNSCC patients. HPV infection was found to deregulate the methylation levels in HPV-positive HNSCC patients"; biomarker analysis of HPV-negative HNSCC patients compared to healthy controls generated a sensitivity of 71% and specificity of 80%, while biomarker analysis of HPV-positive HNSCC patients compared to healthy controls generated a sensitivity of 80% and a specificity of 74% (Lim et al., 2016).

In their overview of non-invasive diagnostic devices in oral oncology, Mascitti et al. (2018) discussed and reviewed the Vizilite® chemiluminescence-based detected device for PMD and OSCC (Zila Pharmaceuticals), VELscope® non-magnifying device for visualization of oral mucosa autofluorescence (LED Medical Diagnostics), Identafi® device for multispectral screening of PMD (StarDEntal-DentalEZ), Microlux/DL™ chemiluminescence-based device (AdDent Inc.), GOCCLES® device for autofluorescence

abnormalities in the oral cavity (Pierrel S.p.A), Orascoptic DK™ chemiluminscence-based device (Orascoptic), and other autofluorescence-based devices like those from Sapphire® PLUS LD (DenMat Holdings), DentLight DOE™ Oral Exam System (DentLight), and ORalID™ 2.0 (Forward Science Technologies). Ultimately, they concluded that there would be "great potential for screening and monitoring lesions. Unfortunately, to date several factors hinder an extensive use of these devices: (1) data do not demonstrate clear superiority of these methods compared to COE; (2) there remains the need for well-designed multicentre prospective studies; (3) these devices exhibit a not negligible interobserver variability limiting their use to clinicians with significant experience in oral pathology." However, in terms of their benefits, "the current evidence suggests that these devices: (1) seem to be useful in assessing lesion margins that must be biopsied and, therefore, may be useful in surgical management; (2) can be used to investigate biological aspects of oral carcinogenesis, leading to more accurate methods for interpreting data from LBDS [light-based detection systems]; (3) can be enhanced with new approaches used to analyse optical imaging data, with the aim to quantify the results obtained; (4) lowering the costs of these devices could indirectly lead to greater attention for oral lesions among both patients and general dental practitioners, allowing in turn to promote a culture of oral cancer prevention; (5) finally, the possibility of implementing LBDS through the use of tissue-marking dyes can in principle allows to develop strategies for the use of nanoparticles. Indeed, nanoparticles can provide molecular targeted imaging, with higher image contrast and resolution" (Mascitti et al., 2018).

Ribeiro et al. (2021) conducted a study aiming to identify prognostic biomarkers for OSCC using a whole genome technology and evaluate their clinical utility. With using array comparative genomic hybridization technology from 62 patients with OSCC, they found that the "chromosomes most commonly altered were 3p, 3q, 5q, 6p, 7q, 8p, 8q, 11q, 15q, 17q, and 18q," with a greater frequency of alterations found on 3p, 3q, 8p, 8q, and 11q. To differentiate between patients with and without metastases or relapses after primary treatment, the researchers identified a genomic signature of genes including *OCLN, CLDN16, SCRIB, IKBKB, PAK2, PIK3CB,* and *YWHAZ*; this rendered an overall accuracy of 79%. An amplification of the *PIK3CB* gene also predicted metastases and relapses in addition to reducing median survival by more than five years. This demonstrated the potential use of genes in developing precision medicine and treating patients with OSCC (Ribeiro et al., 2021).

Guidelines and Recommendations

US Preventive Services Task Force (USPSTF)

In 2013, the USPSTF published final recommendations for screening of oral cancer. The recommendation stated that "the current evidence is insufficient to assess the balance of benefits and harms of screening for oral cancer in asymptomatic adults." The USPSTF also noted that "although there is interest in screening for oral HPV infection, medical and dental organizations do not recommend it" (Moyer, 2014).

National Comprehensive Cancer Network (NCCN)

NCCN clinical practice guidelines on head and neck cancers does not mention the use of adjunctive screening aids based on autofluorescence or tissue reflectance as a management tool (NCCN, 2024). Regarding HPV, the NCCN states that "There are currently no diagnostic tests with regulatory approval" (NCCN, 2024). The NCCN recommends "evaluation of tumor HPV status by use of a surrogate of p16 IHC in all patients diagnosed with an oropharyngeal cancer. Expression of p16 as detected by IHC [immunohistochemistry] is a widely available surrogate biomarker that has very good agreement with HPV status as determined by HPV E6/E7 mRNA expression" (NCCN, 2024).

Additionally, the NCCN states "The performance of various plasma cell-free HPV DNA detection assays (preferably validated per CLIA and CAP regulatory guidelines) for a diagnosis of HPV-positive oropharyngeal cancer against a gold standard of E6/E7 mRNA detection is unknown" (NCCN, 2024).

College of American Pathologists (CAP)

The CAP published guidelines on human papillomavirus testing in head and neck carcinomas. These guidelines state that "For oropharyngeal tissue specimens (ie, noncytology), pathologists should perform HR-HPV [high-risk HPV] testing by surrogate marker p16 IHC" (Lewis et al., 2018).

American Society of Clinical Oncology

An expert panel from the ASCO has "determined that the recommendations from the HPV Testing in Head and Neck Carcinomas guideline, published in 2018, are clear, thorough, and based upon the most relevant scientific evidence. ASCO endorsed the [CAP] guideline and added minor qualifying statements" (Fakhry et al., 2018).

The ASCO states that "It is recommended that HPV tumor status should be determined for newly diagnosed oropharyngeal squamous cell carcinomas. HPV tumor status testing may be performed by surrogate marker p16 immunohistochemistry either on the primary tumor or from cervical nodal metastases only if an oropharyngeal primary tumor is present" (Fakhry et al., 2018).

Regarding diagnosis and management of squamous cell carcinoma of unknown primary (SCCUP) in the head and neck, the ASCO states with a moderate strength recommendation, "High-risk (Fakhry et al.) human papillomavirus (HPV) testing should be done routinely on level II and III SCCUP nodes. Epstein-Barr virus (EBV) testing should be considered on HPV-negative metastases… HR-HPV testing may be done nonroutinely for SCC metastases at other nodal levels when the clinical suspicion is high" (Maghami et al., 2020).

European Head and Neck Society (EHNS)-European Society for Medical Oncology (ESMO)-European Society for Radiotherapy and Oncology (ESTRO)

In 2020, the EHNS, ESMO, and ESTRO released joint clinical practice guidelines for squamous cell carcinoma of the oral cavity, larynx, oropharynx, and hypopharynx. For HPV testing, they recommended that "for SCCHN [squamous cell carcinoma of the head and neck] of unknown primary, p16 and EBER [Epstein-Barr-encoded RNA] are recommended. If p16 staining is positive, another specific HPV test should be carried out to confirm the HPV status [III, A]." p16 measured by immunohistochemistry is validated in use as a surrogate marker for HPV-induced oropharyngeal cancer and prognostic factor for oropharyngeal cancer [I, A] (Machiels et al., 2020)

Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, please visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
81599	Unlisted multianalyte assay with algorithmic analysis
82397	Chemiluminescent assay
87624	Infectious agent detection by nucleic acid (DNA or RNA); Human Papillomavirus (HPV), high-risk types (eg, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68)
87625	Infectious agent detection by nucleic acid (DNA or RNA); Human Papillomavirus (HPV), types 16 and 18 only, includes type 45, if performed
88341	Immunohistochemistry or immunocytochemistry, per specimen; each additional single antibody stain procedure (List separately in addition to code for primary procedure)
88342	Immunohistochemistry or immunocytochemistry, per specimen; initial single antibody stain procedure
0296U	Oncology (oral and/or oropharyngeal cancer), gene expression profiling by RNA sequencing at least 20 molecular features (eg, human and/or microbial mRNA), saliva, algorithm reported as positive or negative for signature associated with malignancy Proprietary test: mRNA CancerDetect™ Lab/Manufacturer: Viome Life Sciences, Inc
0429U	Human papillomavirus (HPV), oropharyngeal swab, 14 high-risk types (ie, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68). Proprietary test: Omnipathology Oropharyngeal HPV PCR Test Lab/Manufacturer: OmniPathology Solutions, Medical Corporation

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

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Revision History

Revision Date	Summary of Changes
09/04/2024	Reviewed and Updated: Updated background, guidelines, and evidence-based scientific references. Literature review necessitated the following changes in coverage criteria: CC1 edited for clarity on the intent of this test (establishing HPV tumor status) for oropharyngeal cancer "1) To establish HPV tumor status for individuals with oropharyngeal squamous cell carcinoma, testing for high-risk HPV with either mRNA expression testing for HPV E6/E7 or immunohistochemistry for p16 expression MEETS COVERAGE CRITERIA." Removed CPT code 87623
12/06/2023	Off-cycle coding modification: Added CPT 0429U (effective date 1/1/2024)

Pancreatic Enzyme Testing for Acute Pancreatitis

Policy Number: AHS – G2153 – Pancreatic Enzyme Testing for Acute Pancreatitis	Prior Policy Name and Number, as applicable:
Initial Presentation Date: 06/01/2018 Effective Date: February 1, 2025	

Policy Description

Pancreatitis is an inflammation of pancreatic tissue and can be either acute or chronic. Pancreatic enzymes, including amylase, lipase, and trypsinogen can be used to monitor the relative health of the pancreatic tissue. Damage to the pancreatic tissue, including pancreatitis, can result in elevated pancreatic enzyme concentrations whereas depressed enzyme levels are associated with exocrine pancreatic insufficiency (Banks et al., 2013; Stevens & Conwell, 2024).

Related Policies

Policy Number	Policy Title
AHS-G2155	General Inflammation Testing
AHS-M2079	Genetic Testing for Hereditary Pancreatitis

Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) For individuals presenting with signs and symptoms of acute pancreatitis (see Note 1), measurement of either serum lipase (preferred) **or** amylase concentration **MEETS COVERAGE CRITERIA**.

2) Measurement of serum lipase and/or amylase concentration **DOES NOT MEET COVERAGE CRITERIA** in any of the following situations:

   a) For individuals with an established diagnosis of acute **or** chronic pancreatitis.

   b) More than once per visit.

   c) For asymptomatic individuals during a general exam without abnormal findings.

3) For the diagnosis, assessment, prognosis, and/or determination of severity of acute pancreatitis, measurement of serum or urine trypsin/trypsinogen/TAP (trypsinogen activation peptide) **DOES NOT MEET COVERAGE CRITERIA**.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

4) For the diagnosis, assessment, prognosis, and/or determination of severity of acute pancreatitis, measurement of the following biomarkers **DOES NOT MEET COVERAGE CRITERIA**:

   a) C-Reactive Protein (CRP)

   b) Interleukin-6 (IL-6)

   c) Interleukin-8 (IL-8)

   d) Procalcitonin

5) For individuals presenting with signs and symptoms of acute pancreatitis (see Note 1), measurement of urinary amylase concentration for the initial diagnosis of acute pancreatitis **DOES NOT MEET COVERAGE CRITERIA**.

6) For all other situations or conditions not described above, measurement of serum lipase and/or amylase **DOES NOT MEET COVERAGE CRITERIA.**

NOTES:

Note 1: Signs and symptoms of acute pancreatitis (Gapp et al., 2023; NIDDK, 2017):

- Mild to severe epigastric pain that begins slowly or suddenly (may spread to the back in some patients)
- Nausea
- Vomiting
- Tender to palpitation of epigastrium
- Abdominal distention
- Hypoactive bowel sounds
- Fever
- Rapid pulse
- Tachypnea
- Hypoxemia

- Hypotension
- Anorexia
- Diarrhea
- Cullen sign
- Grey Turner sign

Table of Terminology

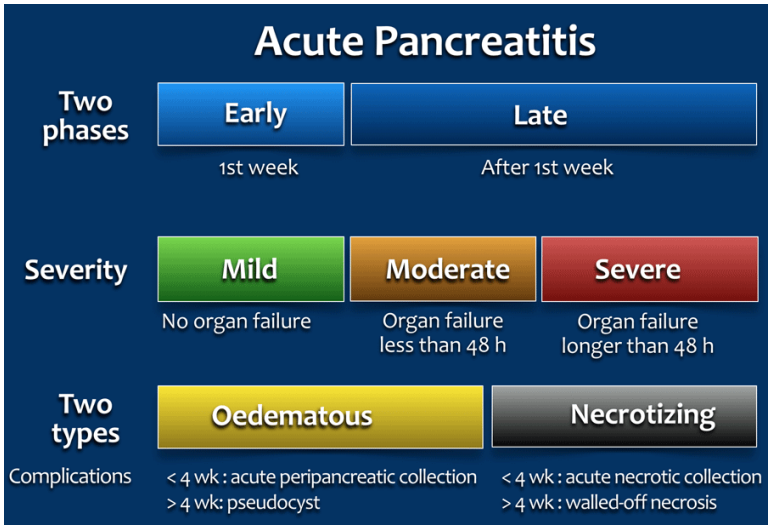
Term	Definition
AACC	American Association for Clinical Chemistry
ABIM	American Board of Internal Medicine
ACCR	Amylase-to-creatinine clearance ratio
ACG	American College of Gastroenterology
AED	Academy For Eating Disorders
AGA	American Gastroenterological Association
AP	Acute pancreatitis
APA	American Pancreatic Association
APA	American Psychiatric Association
APACHE-II	Acute Physiology and Chronic Health Evaluation
ASCP	American Society for Clinical Pathology
AUCs	Area under the curve
BISAP	Bedside index for severity in acute pancreatitis
BUN	Blood urea nitrogen
CADTH	Canadian Agency for Drugs and Technologies in Health
cCRP	Cardiac C-reactive protein
CECT	Contrast-enhanced computed tomography
CLIA '88	Clinical Laboratory Improvement Amendments Of 1988
CMS	Centers For Medicare and Medicaid
CP	Chronic pancreatitis
CPEC	Clinical Practice and Economics Committee
CRP	C-reactive protein
CT	Computed axial tomography
CTSI	Computed axial tomography severity index
ED	Eating disorder
ELISA	Enzyme-linked immunoassay
EPI	Exocrine pancreatic insufficiency
ERCP	Endoscopic retrograde cholangiopancreatography
EUS	Endoscopic ultrasonography
FDA	Food and Drug Administration
GRADE	Grading of recommendations assessment, development, and evaluation
HIV	Human immunodeficiency virus
HMGB1	*High Mobility Group Box 1*
hsCRP	High sensitivity C-reactive protein
HSROC	Hierarchical summary receiver operating characteristics curve
IAP	International Association of Pancreatology
IL-6	Interleukin-6
IL-8	Interleukin-8

LCDs	Local Coverage Determinations
LDH	Lactate dehydrogenase
LDTs	Laboratory-developed tests
MODS	Multiorgan dysfunction syndrome
MRCP	Magnetic resonance cholangiopancreatography
MRI	Magnetic resonance imaging
NASPGHAN	North American Society for Pediatric Gastroenterology, Hepatology and Nutrition Pancreas Committee
NCDs	National Coverage Determinations
PBMCs	Peripheral blood mononuclear cells
PCT	Procalcitonin
PICU	Pediatric intensive care unit
POC	Point of care
RIA	Radioimmunoassay
SIRS	Systemic inflammatory response syndrome
s-isoform	Salivary glands
SPINK1	Serine protease inhibitor Kazal type 1
TAP	Trypsinogen activation peptide
ULN	Upper limit of normal
URL	Upper limit of reference interval
UTDT	Urine trypsinogen dipstick test

Scientific Background

Acute Pancreatitis

Acute pancreatitis (AP) is inflammation of the pancreatic tissue that can range considerably in clinical manifestations. In approximately 80% of individuals, AP clears up completely or shows significant improvement within one to two weeks. However, it can sometimes lead to serious complications and as such, is often treated in a hospital (informedhealth.org, 2021). Due to the lack of consensus in diagnosing, characterizing, and treating AP, an international group of researchers and practitioners convened in Atlanta in 1992 to write a clinically based classification system for AP, which is now commonly referred to as the Atlanta convention or Atlanta classification system (Bradley, 1993). The Atlanta classification system was then revised in 2012 (Banks et al., 2013). For the diagnosis of AP, two of the three following criteria must be present: "(1) *abdominal pain* consistent with acute pancreatitis (acute onset of a persistent, severe, epigastric pain often radiating to the back); (2) serum *lipase activity (or amylase activity) at least three times greater than the upper limit of normal*; and (3) characteristic findings of acute pancreatitis on contrast-enhanced computed tomography (CECT) and less commonly magnetic resonance imaging (Toouli et al.) or transabdominal ultrasonography" (italics emphasized by the manuscript's authors) (Banks et al., 2013). This two-of-three criterion is recommended for diagnostic use by several professional societies (Banks & Freeman, 2006; IAP/APA Working Group, 2013; Tenner et al., 2013). AP can be characterized by two temporal phases, early or late, with degrees of severity ranging from mild (with no organ failure) to moderate (organ failure less than 48 hours) to severe (where persistent organ failure has occurred for more than 48 hours). The two subclasses of AP are edematous AP and necrotizing AP. Edematous AP is due to inflammatory edema with relative homogeneity whereas necrotizing AP displays necrosis of pancreatic and/or peripancreatic tissues (Banks et al., 2013). The figure below from Bollen et al. (2015) outlines the revised Atlanta classification system of AP:



Chronic Pancreatitis

Chronic pancreatitis (ASCP) is also an inflammation of the pancreatic tissue. The two hallmarks of CP are severe abdominal pain and pancreatic insufficiency (Freedman & Forsmark, 2024). Alcohol-induced chronic pancreatitis (or alcohol pancreatitis) accounts for approximately 40-70% of all cases of CP (Klochkov et al., 2023)

The endocrine system is comprised of several glands which secrete hormones directly into the bloodstream to regulate many different bodily functions. On the other hand, the exocrine system is comprised of glands which secrete products through ducts, rather than directly into the bloodstream. CP affects both the endocrine and exocrine functions of the pancreas. Fibrogenesis occurs within the pancreatic tissue due to activation of pancreatic stellate cells by toxins (for example, those from chronic alcohol consumption) or cytokines from necroinflammation. Measuring the serum levels of amylase, lipase, and/or trypsinogen is not helpful in diagnosing CP since not every CP patient experiences acute episodes, the relative serum concentrations may be either decreased or unaffected, and the sensitivities of the tests are not enough to distinguish reduced enzyme levels (Witt et al., 2007). The best method to diagnose CP is to histologically analyze a pancreatic biopsy, but this invasive procedure is not always the most practical so "contrast-enhanced computed tomography is the best imaging modality for diagnosis. Computed tomography may be inconclusive in early stages of the disease, so other modalities such as magnetic resonance imaging, magnetic resonance cholangiopancreatography, or endoscopic ultrasonography with or without biopsy may be used" (Barry, 2018). Previously, ERCP was commonly used to diagnose CP, but the procedure can cause post-ERCP pancreatitis. Genetic factors are also implicated in CP, especially those related to trypsin activity, the serine protease inhibitor SPINK1, and cystic fibrosis (Borowitz et al., 1995; Patel, 2017; Witt et al., 2007).

Amylase

Amylase is an enzyme produced predominantly in the salivary glands (s-isoform) and the pancreas (p-isoform or p-isoamylase) and is responsible for the digestion of polysaccharides, cleaving at the internal 1→4 alpha linkage. Up to 60% of the total serum amylase can be of the s-isoform. The concentration of total serum amylase as well as the pancreatic isoenzyme increase following pancreatic injury or inflammation (Basnayake & Ratnam, 2015; Vege, 2024a). Even though the serum concentration of the pancreatic diagnostic enzymes, including amylase, lipase, elastase, and immunoreactive trypsin all

increase within 24 hours of onset of symptomology, amylase is the first pancreatic enzyme to return to normal levels so the timing of testing is of considerable importance for diagnostic value (Basnayake & Ratnam, 2015; Ventrucci et al., 1987; Yadav et al., 2002). The half-life of amylase is 12 hours since it is excreted by the kidneys, so its clinical value decreases considerably after initial onset of AP. The etiology of the condition can also affect the relative serum amylase concentration. In up to 50% of AP instances due to hypertriglyceridemia (high blood levels of triglycerides), the serum amylase concentration falls into the normal range, and normal concentrations of amylase has been reported in cases of alcohol-induced AP (Basnayake & Ratnam, 2015; Quinlan, 2014); in fact, one study shows that 58% of the cases of normoamylasemic AP was associated with alcohol use (Clavien et al., 1989). Elevated serum amylase concentrations also can occur in conditions other than AP, including hyperamylasemia (excess amylase in the blood) due to drug exposure (Ceylan et al., 2016; Liu et al., 2016), bulimia nervosa (Wolfe et al., 2011), leptospirosis (Herrmann-Storck et al., 2010), and macroamylasemia (Vege, 2024a). Serum amylase levels are often significantly elevated in individuals with bulimia nervosa due to recurrent binge eating episodes (Wolfe et al., 2011).

Macroamylasemia is a condition where the amylase concentration increases due to the formation of macroamylases, complexes of amylase with immunoglobulins and/or polysaccharides. Macroamylasemia is associated with other disease pathologies, "including celiac disease, HIV infection, lymphoma, ulcerative colitis, rheumatoid arthritis, and monoclonal gammopathy". Suspected macroamylasemia in instances of isolated amylase elevation can be confirmed by measuring the amylase-to-creatinine clearance ratio (ACCR) since macroamylase complexes are too large to be adequately filtered. Normal values range from three to four percent with values of less than one percent supporting the diagnosis of macroamylasemia. ACCR itself is not a good indicator of AP since low ACCR is also exhibited in diabetic ketoacidosis and severe burns (Vege, 2024a). Hyperamylasemia is also seen in other extrapancreatic conditions, such as appendicitis, salivary disease, gynecologic disease, extra-pancreatic tumors, and gastrointestinal disease (Terui et al., 2013; Vege, 2024a). Gullo's Syndrome (or benign pancreatic hyperenzymemia) is a rare condition that also exhibits high serum concentrations of pancreatic enzymes without showing other signs of pancreatitis (Kumar et al., 2016). No correlation has been found between the concentration of serum amylase and the severity or prognosis of AP (Lippi et al., 2012).

Urinary amylase and peritoneal amylase concentrations can also be measured. Rompianesi et al. (2017) reviewed the use of urinary amylase and trypsinogen as compared to serum amylase and serum lipase testing. The authors note that "with regard to urinary amylase, there is no clear-cut level beyond which someone with abdominal pain is considered to have acute pancreatitis". Three studies regarding urinary amylase were reviewed —each with 134-218 participants—and used the hierarchical summary receiver operating characteristics curve (HSROC) analysis to compare the accuracy of the studies. Results showed that "the models did not converge" and the authors concluded that "we were therefore unable to formally compare the diagnostic performance of the different tests" (Rompianesi et al., 2017).

Another study investigated the use of peritoneal amylase concentrations for diagnostic measures and discovered that patients with intra-abdominal peritonitis had a mean peritoneal amylase concentration of 816 U/L (142-1746 U/L range), patients with pancreatitis had a mean concentration of 550 U/L (100-1140 U/L range), and patients with other "typical infectious peritonitis" had a mean concentration of 11.1 U/L (0-90 U/L range). Conclusions state "that peritoneal fluid amylase levels were helpful in the differential diagnosis of peritonitis in these patients" and that levels >100 U/L "differentiated those patients with other intra-abdominal causes of peritonitis from those with typical infectious peritonitis" (Burkart et al., 1991). The authors do not state if intraperitoneal amylase is specifically useful in diagnosing AP.

Liu et al. (2021) conducted a retrospective cohort study to evaluate whether serum amylase and lipase could serve as a biomarker to predict pancreatic injury in 79 critically ill children who died of different causes. Through autopsy investigation, the subjects were divided into pancreatic injury group and non-pancreatic injury group. Forty-one patients (51.9%) exhibited pathological changes of pancreatic injury. Levels of lactate, erythrocyte sedimentation rate, alanine transaminase, aspartate transaminase, and troponin-I in the pancreatic injury group were significantly higher than that in the noninjury group. "Multivariable logistic regression analysis showed that serum amylase, serum lipase, and septic shock were significantly associated with the occurrence rate of pancreatic injury". Therefore, the authors conclude that "serum amylase and lipase could serve as independent biomarkers to predict pancreatic injury in critically ill children" (Liu et al., 2021).

In a prospective case control study, Judal et al. (2022) investigated urinary amylase levels for diagnosis of acute pancreatitis. One major challenge with measurement of serum amylase is its short half-life which returns to normal levels within a short period of time. This study enrolled 100 patients (50 healthy and 50 with acute pancreatitis) who were measured for serum amylase, serum lipase, and urinary amylase. There was a statistically significant increase in the serum amylase, lipase, and urinary amylase mean values of patients with AP. "Serum amylase had the highest sensitivity (100%) and serum lipase had the highest specificity (96.53%). The sensitivity and specificity of urinary amylase was found to be 97.25% and 91.47% respectively" (Judal et al., 2022). The authors conclude that urinary amylase is a convenient and sensitive test for diagnosis.

Ryholt et al. (2024) conducted a retrospective study with data collected throughout 2020 to "assess the utilization of appropriate laboratory testing related to the diagnosis of acute pancreatitis." The authors were particularly interested in the overuse of amylase testing or amylase and lipase testing together when lipase testing alone would have been sufficient for AP diagnosis. Overall, 2567 (9.3%) of all amylase and lipase tests were determined to be unnecessary, an estimated $128,350 in total cost savings if eliminated. Of the unnecessary tests, 1881 (73.2%) were amylase tests and 686 (26.7%) were lipases tests. The authors also note that "an analysis of test-ordering behavior by providers revealed that 81.5% of all unnecessary tests were ordered by MDs." The authors conclude that the "study demonstrated that amylase and lipase tests have been overutilized in the diagnosis of acute pancreatitis" (Ryholt et al., 2024).

Lipase (Pancreatic Lipase or Pancreatic Triacylglycerol Lipase)

Pancreatic lipase or triacylglycerol lipase (herein referred to as "lipase") is an enzyme responsible for hydrolyzing triglycerides to aid in the digestion of fats. Like amylase, lipase concentration increases shortly after pancreatic injury (within three to six hours). However, contrary to amylase, serum lipase concentrations remain elevated for one to two weeks after initial onset of AP since lipase can be reabsorbed by the kidney tubules (Lippi et al., 2012). Moreover, the pancreatic lipase concentration is 100-fold higher than the concentration of other forms of lipases found in other tissues such as the duodenum and stomach (Basnayake & Ratnam, 2015). Both the sensitivity and the specificity of lipase in laboratory testing of AP are higher than that of amylase (Yadav et al., 2002). A study by Coffey et al. (2013) found "an odds ratio of 7.1 (95% confidence interval 2.5-20.5; $P<0.001$) for developing severe AP" in patients ages 18 or younger when the serum lipase concentration is at least 7-fold higher than upper limit of normal. However, in general, elevated serum lipase concentration is not used to determine the severity or prognosis of AP (Ismail & Bhayana, 2017). Hyperlipasemia can also occur in other conditions such as Gullo's Syndrome (Kumar et al., 2016). The use of lipase to determine etiology of AP is of debate. A study by Levy et al. (2005) reports that lipase alone cannot be used to determine biliary cause of AP whereas other studies have indicated that a ratio of lipase-to-amylase concentrations ranging from 2:1

to more than 5:1 can be indicative of alcohol-induced AP (Gumaste et al., 1991; Ismail & Bhayana, 2017; Pacheco & Oliveira, 2007; Tenner & Steinberg, 1992).

The review by Ismail and Bhayana (2017) included a summary table (Table 1 below) comparing various studies concerning the use of amylase and lipase for diagnosis of AP as well as a table (Table 2 below) comparing the cost implication of the elimination of double-testing for AP.

Table 1: Summary of numerous studies comparing lipase against amylase (URL – Upper Limit of Reference interval, AP – Acute Pancreatitis).

Design and reference	**Participant (patients with abdominal pain/AP)**	**Threshold**	**Results**		**Conclusion**
			Serum lipase	**Serum amylase**	
Prospective study [56]	384/60	Two times URL	Diagnostic accuracy and efficiency are > 95% for both		No difference between amylase and lipase in diagnosing AP
Prospective study [57]	306/48	Serum lipase > 208 U/L Serum amylase > 110 U/L	92% sensitivity 87% specificity 94% Diagnostic accuracy	93% sensitivity 87% specificity 91% Diagnostic accuracy	Both tests are associated with AP, but serum lipase is better than amylase
Prospective study [58]	328/51	Serum lipase: > 208 U/L (Day 1) > 216 U/L (Day 3) Serum amylase: > 176 U/L > 126 U/L (Day 3)	Day 1: 64 % Sensitivity 97% Specificity Day 3: 55% Sensitivity 84% Specificity	Day 1: 45 % Sensitivity 97% Specificity Day 3: 35% Sensitivity 92% Specificity	Serum lipase is better at diagnosing early and late AP
Retrospective study [63]	17,531/320 *49 had elevated lipase only	Serum lipase > 208 U/L Serum amylase > 114 U/L	90.3% Sensitivity 93.6% Specificity	78.7% Sensitivity 92.6% Specificity	Serum lipase is more accurate marker for AP
Cohort study [2]	1,520/44	Three times URL	64% Sensitivity 97% Specificity	50% Sensitivity 99% Specificity	Serum lipase is preferable to use in comparison to amylase alone or both tests
Retrospective study [59]	3451/34 *33 patients had elevated amylase and 50 had	Three or more times URL	95.5% Sensitivity 99.2% Specificity	63.6% Sensitivity 99.4% Specificity	Both enzymes have good accuracy, but lipase is more

	elevated lipase only				sensitive than amylase
Cohort study [60]	151/117 *6 patients with gallstone-induced and 5 patients with alcohol-induced AP had elevated lipase only	Three times URL	96.6% Sensitivity 99.4% Specificity	78.6% Sensitivity 99.1% Specificity	Lipase is more sensitive in diagnosing AP and using it alone would present a substantial cost saving on health care system
Prospective study [61]	476/154 *58 patients had a normal amylase level	Three times URL	91% Sensitivity 92% Specificity	62% Sensitivity 93% Specificity	Lipase is more sensitive than amylase and should replace amylase in diagnosis of AP
Cohort study [62]	50/42 *8 patients had elevated lipase only	Three times URL	100% Sensitivity	78.6% Sensitivity	Lipase is a better choice than amylase in diagnosis of AP

This table is a list of individual studies examining the specificity and sensitive of serum lipase and serum amylase in diagnosing AP. In each of the listed studies except one, the authors concluded that serum lipase is better than serum amylase for AP. The only outlier used a lower threshold in considering enzyme elevation; in particular, two times the upper limit of reference interval (URL) was used whereas the Atlanta classification system recommends at least three times the URL to determine enzyme elevation (Ismail & Bhayana, 2017).

Table 2: Summary of studies exploring the cost implication associated with eliminating amylase test.

Design and Reference	Costs	Volume of test	Results
Cohort study (UK) [2]	Amylase costs £1.94 Lipase cost £2.50	1383 request for 62 days costing £6136 for both tests	Testing lipase only will result in cost saving
Cohort study (UK) [60]	Single amylase or lipase cost about £0.69 each Cost of both measured together were £0.99.	2979 requests costing £2949.21	Measuring lipase would save health care system an estimate of £893.70 per year
Prospective study (US) [71]	Patients charged $35 for either lipase or amylase	618 co-ordered both lipase and amylase	Amylase test was removed from common order sets in the electronic medical record Reduced the co-ordering of lipase and amylase to 294 Overall saving of $135,000 per year

This table specifically outlines studies that compared the financial cost of the serum amylase and serum lipase tests for diagnosing AP. All three studies show cost savings if only lipase concentration is used. In fact, one study by researchers in Pennsylvania resulted in the removal of the amylase test "from common order sets in the electronic medical record" (Ismail & Bhayana, 2017).

Furey et al. (2020) compared amylase and lipase ordering patterns for patients with AP. A total of 438 individuals were included in this study. The researchers noted that "All patients had at least one lipase ordered during their admission, and only 51 patients (12%) had at least one amylase ordered. On average, lipase was elevated 5 times higher above its respective upper reference limit than amylase at admission" (Furey et al., 2020). Further, patients undergoing a laparoscopic cholecystectomy (gallbladder removal) were more likely to have amylase ordered. These results showed that in 88% of patients with AP, amylase measurement was not necessary; moreover, "Of patients for whom amylase was ordered, it was common for these patients to be those referred to surgical procedures, possibly because amylase normalization may be documented faster than that of lipase" (Furey et al., 2020).

In a retrospective cross-sectional study by El Halabi et al. (2019), the clinical utility and economic burden of routine serum lipase examination in the emergency department was observed. From 24,133 adult patients admitted within a 12-month period, serum lipase levels were ordered for 4,976 (20.6%) patients. Of those 614 (12.4%) who had abnormal lipase levels, 130 of those patients were above the diagnostic threshold for acute pancreatitis (>3 times the ULN) and 75 patients had confirmed diagnosis of acute pancreatitis. In total, 1,890 patients had normal no abdominal pain or history of acute pancreatitis, but 251 of these patients were tested for lipase levels, leading to a total cost of $51,030. These results triggered unneeded cross-sectional abdominal imaging in 61 patients and unwarranted gastroenterology consultation in three patients, leading to an additional charge of $28,975. The authors conclude that "serum lipase is widely overutilised in the emergency setting resulting in unnecessary expenses and investigations" (El Halabi et al., 2019).

Liu et al. (2021) studied the use of serum amylase and lipase for the prediction of pancreatic injury in critically ill children admitted to the PICU. Seventy-nine children who died from different cases were studied from autopsy and it was found that 41 of these patients had pathological signs of pancreatic injury. Multivariable logistic regression analysis showed that serum amylase, serum lipase, and septic shock were significantly associated with the occurrence rate of pancreatic injury. Serum amylase was measured with 53.7% sensitivity, 81.6% specificity, cut off value of 97.5, and AUC of 0.731. Serum lipase was measured with 36.6% sensitivity, 92.1% specificity, cut off value of 61.1, and AUC of 0.727. The authors conclude that "serum amylase and lipase could serve as independent biomarkers to predict pancreatic injury in critically ill children" (Liu et al., 2021).

Ritter. J et al. (2019) showed that for individuals with acute pancreatitis experiencing a hospital stay, there was no difference in disease severity between individuals who had repeat lipase and/or amylase testing and those who did not have repeat testing. They found that approximately "one-third of inpatient encounters with at least one elevated amylase or lipase test continued with repeat testing with as many as 25 additional tests after the initial elevated test result. The mean number of unnecessary additional serial tests was 2.8 and 2.4 for amylase and lipase, respectively, consistent with the tests being ordered each hospital day, given a 3-day nationwide average inpatient stay for acute pancreatitis" (Ritter. J et al., 2019). According to their findings, "ambulatory settings had the highest rates of concurrent testing while emergency departments had the lowest" (Ritter. J et al., 2019). While the cost of unnecessary serial and concurrent amylase/lipase tests are relatively small when considering the entire health system, based on their findings, they estimated that the national impact of these two tests could be as much as $5.8 million in variable costs alone. They concluded that unnecessary laboratory testing

remains a problem despite evidence-based guidelines and programs that have been designed to reduce and eliminate it (Ritter. J et al., 2019).

Trypsin/Trypsinogen/TAP

Trypsin is a protease produced by the pancreatic acinar cells. Trypsin is first synthesized in its zymogen form, trypsinogen, which has its N-terminus cleaved to form the mature trypsin. Pancreatitis can result in blockage of the release of the proteases while their synthesis continues. This increase in both intracellular trypsinogen and cathepsin B, an enzyme that can cleave the trypsinogen activation peptide (TAP) from the zymogen to form mature trypsin, results in a premature intrapancreatic activation of trypsin. This triggers a release of both trypsin and TAP extracellularly into the serum and surrounding peripancreatic tissue. Due to the proteolytic nature of trypsin, this response can result in degradation of both the pancreatic and peripancreatic tissues (i.e., necrotizing AP) (Vege, 2024c; Yadav et al., 2002). Trypsin activity "is critical for the severity of both acute and chronic pancreatitis" (Zhan et al., 2019). When the intracellular activity of trypsin escalates, an increase is also reflected in the number of pancreatitis cases overall, as well as in the severity of these cases (Sendler & Lerch, 2020).

Since trypsinogen is readily excreted, a urine trypsinogen-2 dipstick test has been developed (Actim Pancreatitis test strip from Medix Biochemica), which has a reported specificity of 85% for severe AP within 24 hours of hospital admission (Lempinen et al., 2001). Another study reported that the trypsinogen-2 dipstick test has a specificity of 95% and a sensitivity of 94% for AP, which is higher than a comparable urine test for amylase (Kemppainen et al., 1997). As of 2023, the FDA has not approved the use of the trypsinogen-2 dipstick test for the detection or diagnosis of AP. The quality control review of the clinical trial is underway in the United States (Eastler, 2023). The use of TAP for either a diagnostic or prognostic tool is of debate (Lippi et al., 2012).

The study by Neoptolemos et al. (2000) reported that a urinary TAP assay had a 73% specificity for AP. However, another study using a serum TAP methodology reported a 23.5% sensitivity and 91.7% specificity for AP and concluded that "TAP is of limited value in assessing the diagnosis and the severity of acute pancreatic damage" (Pezzilli et al., 2004).

Yasuda et al. (2019) completed a multicenter study in Japan which measured the usefulness of the rapid urinary trypsinogen-2 dipstick test and levels of urinary trypsinogen-2 and TAP concentration as prognostic tools for AP. A total of 94 patients participated in this study from 17 medical institutions between April 2009 and December 2012. The researchers determined that "The trypsinogen-2 dipstick test was positive in 57 of 78 patients with acute pancreatitis (sensitivity, 73.1%) and in 6 of 16 patients with abdominal pain but without any evidence of acute pancreatitis (specificity, 62.5%)" (Yasuda et al., 2019). Further, both TAP and urinary trypsinogen-2 levels were significantly higher in patients with extra-pancreatic inflammation. The authors concluded that the urinary trypsinogen-2 dipstick test is a useful tool for AP diagnoses.

Simha et al. (2021) studied the utility of POC urine trypsinogen dipstick test for diagnosing AP in an emergency unit. Urine trypsinogen dipstick test (UTDT) was performed in 187 patients in which 90 patients had AP. UTDT was positive in 61 (67.7%) of the 90 AP patients. In the 97 non pancreatitis cases, UTDT was positive in nine of those cases (9.3%). The sensitivity and specificity of UTDT for acute pancreatitis was 67.8% and 90.7%, respectively. The authors conclude that although it is a great and convenient possibility as a POC test, "the low sensitivity of UTDT could be a concern with its routine use" (Simha et al., 2021).

Other Biochemical Markers (CRP, Procalcitonin, IL-6, IL-8)

Acute pancreatitis results in the activation of the immune system. Specific markers including C-reactive protein (CRP), procalcitonin, interleukin-6 (IL-6), and interleukin-8 (IL-8) have been linked to AP (Toouli et al., 2002; Vege, 2024b; Yadav et al., 2002). CRP is a nonspecific marker for inflammation that takes 48-72 hours to reach maximal concentration after initial onset of AP but is reported to have a specificity of 93% in detecting pancreatic necrosis. CRP can be used in monitoring the severity of AP; however, imaging techniques, including CT, and evaluative tools, such as the APACHE-II (acute physiology and chronic health evaluation) test, are preferred methods (IAP/APA Working Group, 2013; Quinlan, 2014).

Procalcitonin is the inactive precursor of the hormone calcitonin. Like CRP, procalcitonin has been linked to inflammatory responses, especially in response to infections and sepsis. Procalcitonin levels are elevated in AP and are significantly elevated (≥3.5 ng/mL for at least two consecutive days) in cases of AP associated with multiorgan dysfunction syndrome (MODS) (Rau et al., 2007). Moreover, the elevated procalcitonin levels decrease upon treatment for AP; "however, further research is needed in order to understand how these biomarkers can help to monitor inflammatory responses in AP" (Simsek et al., 2018).

The concentration of inflammatory cytokines IL-6 and IL-8 become elevated in AP with a maximal peak within the first 24 hours after initial onset of AP (Yadav et al., 2002). One study by Jakkampudi et al. (2017) shows that IL-6 and IL-8 are released in a time-dependent manner after injury to the pancreatic acinar cells. This, in turn, activated the peripheral blood mononuclear cells (PBMCs), which propagate acinar cell apoptosis that results in further release of cytokines to increase the likelihood of additional cellular damage.

A study conducted by Khanna et al. (2013) compares the use of biochemical markers, such as CRP, IL-6, and procalcitonin, in predicting the severity of AP and necrosis to that of the clinically used evaluative tools, including the Glasgow score and APACHE-II test. Their results indicate that CRP has a sensitivity and specificity of 86.2% and 100%, respectively, for severe AP and a sensitivity and specificity of 100% and 81.4%, respectively, for pancreatic necrosis. These scores are better than those reported for the clinical evaluative tools (see table below). IL-6 also shows an increase in both sensitivity and specificity; however, the values for procalcitonin are considerably lower than either CRP or IL-6 in all parameters (Khanna et al., 2013).

Data from	***Severe AP***		***Pancreatic necrosis***	
(Khanna et al., 2013)	*Sensitivity*	*Specificity*	*Sensitivity*	*Specificity*
Glasgow	*71.0*	*78.0*	*64.7*	*63.6*
APACHE-II	*80.6*	*82.9*	*64.7*	*61.8*
CRP	*86.2*	*100*	*100*	*81.4*
IL-6	*93.1*	*96.8*	*94.1*	*72.1*
Procalcitonin	*86.4*	*75.0*	*78.6*	*53.6*

Another study by Hagjer and Kumar (2018) compared the efficacy of the bedside index for severity in acute pancreatitis (BISAP) scoring system to CRP and procalcitonin shows that CRP is not as accurate for prognostication as BISAP. BISAP has AUCs for predicting severe AP and death of 0.875 and 0.740, respectively, as compared to the scores of CRP (0.755 and 0.693, respectively). Procalcitonin, on the

other hand, had values of 0.940 and 0.769 for predicting severe AP and death, respectively. The authors concluded that it "is a promising inflammatory marker with prediction rates similar to BISAP" (Hagjer & Kumar, 2018).

Li et al. (2018) completed a meta-analysis to determine the relationship between high mobility group box 1 (HMGB1), interleukin-6 (IL-6) and AP. HMGB1 protein is a nuclear protein with several different purposes depending on its location (Yang et al., 2015). These researchers analyzed data from 27 different studies comprised of 1908 of participants (896 with mild AP, 700 with severe AP and 312 healthy controls). Overall, serum HMGB1 and IL-6 levels were higher in patients with both severe and mild AP compared to controls; further, and serum HMGB1 and IL-6 levels were significantly higher in patients with severe AP than mild AP (Li et al., 2018). The authors concluded that serum HMGB1 and IL-6 levels "might be used as effective indicators for pancreatic lesions as well as the degree of inflammatory response" and that both HMGB1 and IL-6 are closely correlated with pancreatitis severity.

Tian et al. (2020) studied the diagnostic value of C-reactive protein (CRP), procalcitonin (PCT), IL-6, and lactate dehydrogenase (LDH) in patients with severe acute pancreatitis. A total of 153 patients were divided into the mild acute pancreatitis group (81) and severe pancreatitis group (72). Significant differences in the values of these enzymes were found between both groups. The sensitivity, specificity, and AUC were determined as seen in the chart below. The AUC of combined detection of CRP, PCT, IL-6 and LDH was 0.989. The authors conclude that "the combined detection of CRP, PCT, IL-6 and LDH has a high diagnostic value for judging the severity of acute pancreatitis" (Tian et al., 2020).

Enzyme	Sensitivity	Specificity	AUC
CRP	55.6%	73%	0.637
PCT	77.8%	94%	0.929
IL-6	80.2%	85%	0.886
LDH	82.7%	96%	0.919

In a retrospective cohort study, Wei et al. (2022) investigated the predictive value of serum cholinesterase (ChE) in the mortality of acute pancreatitis. A total of 692 patients were enrolled in the study and were divided into the ChE-low group (378 patients) or ChE-normal group (314 patients). Mortality was significantly different in two groups (10.3% in ChE-low vs. 0.0% ChE- normal) and organ failure also differed (46.6% ChE-low vs. 8.6% ChE-normal). The area under the curve of serum ChE was 0.875 and 0.803 for mortality and organ failure, respectively. The authors conclude that "lower level of serum ChE was independently associated with the severity and mortality of AP" (Wei et al., 2022).

Guidelines and Recommendations

International Association of Pancreatology (IAP/APA Working Group) and the American Pancreatic Association (APA)

In 2012, a joint conference between the International Association of Pancreatology (IAP/APA Working Group) and the American Pancreatic Association (APA) convened to address the guidelines for the management of acute pancreatitis. This conference made their recommendations using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) system. The IAP/APA Working Group (2013) are detailed with 38 recommendations covering 12 different topics, ranging from diagnosis to predicting severity of disease to timing of treatments. As concerning the diagnosis and

etiology of AP, the associations conclude with "GRADE 1B, strong agreement" that the definition of AP follow the Atlanta classification system where at least two of the following three criteria are evident—the clinical manifestation of upper abdominal pain, the laboratory testing of serum amylase or serum lipase where levels are more than three times the upper limit of normal values, and/or the affirmation of pancreatitis using imaging methods (IAP/APA Working Group, 2013). IAP/APA Working Group (2013) specifically did not include the trypsinogen-2 dipstick test in their recommendations "because of its presumed limited availability". One question addressed by the committee was the continuation of oral feeding being withheld for patients until the lab serum tests returned within normal values. With a GRADE 2B, strong agreement finding, they conclude that "it is not necessary to wait until pain or laboratory abnormalities completely resolve before restarting oral feeding" (IAP/APA Working Group, 2013). No specific discussion on the preference of either serum amylase or lipase is included within the guidelines as well as no discussion of the use of either serum test beyond initial diagnosis of AP (i.e., no continual testing for disease monitoring is included). Furthermore, no discussion concerning the use of urinary or peritoneal amylase concentrations for AP.

With regards to CRP and/or procalcitonin, the IAP/APA does not address the topic in any detail. As part of IAP/APA Working Group (2013) recommendation (GRADE 2B) concerning the best score or marker to predict the severity of AP, they state "that there are many different predictive scoring systems for acute pancreatitis..., including single serum markers (C-reactive protein, hematocrit, procalcitonin, blood urea nitrogen), but none of these are clearly superior or inferior to (persistent) SIRS", which is Systemic inflammatory response syndrome. Moreover, in response to their recommendation for admission to an intensive care unit in AP (GRADE 1C), they state that "the routine use of single markers, such as CRP, hematocrit, BUN or procalcitonin alone to triage patients to an intensive care setting is not recommended" (IAP/APA Working Group, 2013).

American Gastroenterological Association (AGA)

The Clinical Practice and Economics Committee (CPEC) of the American Gastroenterological Association (AGA) Institute released the AGA Institute Medical Position Statement on Acute Pancreatitis as approved by the AGA Institute Governing Board in 2007 to address differences in the recommendations of various national and international societies concerning AP. Within their recommendations, Baillie (2007) address the necessity of timeliness in the applicability of serum amylase and/or serum lipase testing. Per their recommendations, either serum amylase or serum lipase should be tested within 48 hours of admission. AP is consistent with amylase or lipase levels greater than three times the upper limit of the normal value. Baillie (2007) specifically state that the "elevation of lipase levels is somewhat more specific and is thus preferred". The AGA guidelines do not address the use of either urinary or peritoneal concentrations of amylase in AP. Also, any patient presenting symptoms of unexplained multiorgan failure or systemic inflammatory response syndrome should be tested for a possible AP diagnosis. Concerning etiology of the phenotype, they suggest that upon admission, "all patients should have serum obtained for measurement of amylase or lipase level, triglyceride level, calcium level, and liver chemistries" (Baillie, 2007). Invasive evaluation, such as endoscopic retrograde cholangiopancreatography (ERCP), should be avoided for patients with a single occurrence of AP. The only mention of CRP in their guidelines is in the section concerning the severity (and not the diagnosis of) AP. "Laboratory tests may be used as an adjunct to clinical judgment, multiple factors scoring systems, and CT to guide clinical triage decisions. A serum C-reactive protein level >150 mg/L at 48 hours after disease onset is preferred" (Baillie, 2007).

In 2018, the AGA published guidelines on the initial management of AP. These guidelines state that "the diagnosis of AP requires at least 2 of the following features: characteristic abdominal pain; biochemical

evidence of pancreatitis (ie, amylase or lipase elevated >3 times the upper limit of normal); and/or radiographic evidence of pancreatitis on cross-sectional imaging" (Crockett et al., 2018).

The AGA Clinical Practice Update on the Epidemiology, Evaluation, and Management of Exocrine Pancreatic Insufficiency (EPI) advise that exocrine pancreatic insufficiency "should be suspected in patients with high-risk clinical conditions, such as chronic pancreatitis, relapsing acute pancreatitis, pancreatic ductal adenocarcinoma, cystic fibrosis, and previous pancreatic surgery. . . fecal elastase test is the most appropriate initial test and must be performed on a semi-solid or solid stool specimen. A fecal elastase level <100 µg/g of stool provides good evidence of EPI, and levels of 100–200 µg/g are indeterminate for EPI" (Whitcomb et al., 2023).

American College of Gastroenterology (ACG)

The ACG released guidelines concerning AP in both 2006 and 2013. Both sets of guidelines recommend the use of the Atlanta classification system regarding the threshold of either serum amylase or serum lipase levels in the diagnosis of AP (i.e., greater than three times the upper limit of normal range). Both sets of guideline's state that the standard diagnosis is meeting at least two of the three criteria as stated in the revised Atlanta classification system (Banks & Freeman, 2006; Tenner et al., 2013).

The 2006 guidelines discuss the differences between serum amylase and lipase in greater detail. First, although both enzymes can be elevated in AP, the sensitivity and half-life of lipase are more amenable for diagnosis since the levels of lipase remain elevated longer than those of amylase. These guidelines also make note that "it is usually not necessary to measure both serum amylase and lipase" and that "the daily measurement of serum amylase or lipase after the diagnosis of acute pancreatitis has limited value in assessing the clinical progress of the illness". These guidelines discuss the possibility of elevated amylase levels due to causes other than AP, including but not limited to macroamylasemia, whereas the serum levels of lipase are unaffected by these conditions (Banks & Freeman, 2006).

The 2013 guidelines do not explicitly state a preference of the serum lipase over serum amylase test in the diagnosis of AP. They also state that lipase levels can be elevated in macrolipasemia as well as certain nonpancreatic conditions, "such as renal disease, appendicitis, cholecystitis, and so on". Neither set of guidelines address the use of either urinary or peritoneal amylase in AP. The 2006 guidelines list other diagnostic tests, including the trypsin/trypsinogen tests as well as serum amyloid A and calcitonin but do not address them further given their limited availability at that time whereas the 2013 guidelines state that, even though other enzymes can be used for diagnostics, "none seems to offer better diagnostic value than those of serum amylase and lipase". They even state that "even the acute-phase reactant C-reactive protein (CRP) the most widely studied inflammatory marker in AP, is not practical as it takes 72h to become accurate" (Tenner et al., 2013).

American Board of Internal Medicine (ABIM), American Society for Clinical Pathology (ASCP) and Choosing Wisely

In 2020, the ASCP, along with Choosing Wisely and the ABIM Foundation, published a brochure titled *Thirty Things Physicians and Patients Should Question.* This brochure includes the following recommendation:

"Do not test for amylase in cases of suspected acute pancreatitis. Instead, test for lipase.

Amylase and lipase are digestive enzymes normally released from the acinar cells of the exocrine pancreas into the duodenum. Following injury to the pancreas, these enzymes are released into the

circulation. While amylase is cleared in the urine, lipase is reabsorbed back into the circulation. In cases of acute pancreatitis, serum activity for both enzymes are greatly increased.

Serum lipase is now the preferred test due to its improved sensitivity, particularly in alcohol-induced pancreatitis. Its prolonged elevation creates a wider diagnostic window than amylase. In acute pancreatitis, amylase can rise rapidly within 3–6 hours of the onset of symptoms and may remain elevated for up to five days. Lipase, however, usually peaks at 24 hours with serum concentrations remaining elevated for 8–14 days. This means it is far more useful than amylase when the clinical presentation or testing has been delayed for more than 24 hours.

Current guidelines and recommendations indicate that lipase should be preferred over total and pancreatic amylase for the initial diagnosis of acute pancreatitis and that the assessment should not be repeated over time to monitor disease prognosis. Repeat testing should be considered only when the patient has signs and symptoms of persisting pancreatic or peripancreatic inflammation, blockage of the pancreatic duct or development of a pseudocyst. Testing both amylase and lipase is generally discouraged because it increases costs while only marginally improving diagnostic efficiency compared to either marker alone" (ASCP, 2020).

North American Society for Pediatric Gastroenterology, Hepatology and Nutrition Pancreas Committee (NASPGHAN)

The NASPGHAN states that the primary biomarkers used to diagnose AP are serum lipase and amylase and note that "a serum lipase or amylase level of at least 3 times the upper limit of normal is considered consistent with pancreatitis". Further, NASPGHAN acknowledges that other biomarkers for diagnosis and management of AP have been investigated, but none are prominent and "many have yet to be validated for general clinical use" (NASPGHAN, 2018).

American Psychiatric Association (APA)

The APA published a practice guideline in 2023 for the treatment of patients with eating disorders. In this guideline, pancreatitis (in adults and in adolescents) is just one of a set of factors that supports medical hospitalization or hospitalization on a specialized eating disorder unit.

Also, the APA notes that "serum amylase levels, specifically levels of salivary amylase, may be elevated in patients who self-induce vomiting. With starvation and with renourishment, elevations in serum lipase can be seen but generally do not require intervention" (APA, 2023).

Academy for Eating Disorders (AED) Medical Care Standards Committee

The AED has published a guide to medical care for eating disorders. A table is included in these guidelines which is titled *Diagnostic Tests Indicated for All Patients with A Suspected ED* [eating disorder]. In a subcategory, titled *Criteria Supportive of Hospitalization for Acute Medical Stabilization*, these guidelines mention that "acute medical complications of malnutrition" including pancreatitis may occur (AED, 2021).

The American Association for Clinical Chemistry

The American Association for Clinical Chemistry released recommendations for amylase testing in diagnosis and management of acute pancreatitis. The AACC provides the following recommendations:

- "For diagnosis and management of acute pancreatitis, do not order this test if serum lipase test is available.
- May be considered for the diagnosis and monitoring of chronic pancreatitis and other pancreatic diseases."

The AACC does mention that "the test is not specific for pancreatitis and may be elevated due to other, non-pancreatic causes (such as acute cholecystitis, inflammatory bowel disease, intestinal obstruction, certain cancers, salivary disease, macroamylasemia, etc.)".

1. The AACC further states to "consider ordering this test when serum lipase is not available as a stat test and the patient presents with a sudden onset of abdominal pain with nausea and vomiting, fever, hypotension, and abdominal distension

" and that "testing both amylase and lipase should be discouraged because it increases costs while only marginally improving diagnostic efficiency compared to lipase alone" (AACC, 2023).

Canadian Agency for Drugs and Technologies in Health (CADTH)

The CADTH has published an advisory panel guidance on minimum retesting intervals for lab tests. They identify the following key issues:

- "Lab test overuse can contribute to further unnecessary follow-up and testing, negative patient experiences, potentially inappropriate treatments, and the inefficient use of health care resources. One review of lab testing in Canada found that around 22% of blood tests were likely unnecessary.
- One strategy to address lab test overuse is to establish minimal retesting intervals that can be implemented in medical laboratories to help identify and manage potentially inappropriate lab test requests.
- Minimum retesting intervals suggest the minimum time before a test should be repeated based on the biochemical properties of the test and the clinical situation in which it is used. They are intended to inform clinical decisions about repeat testing" (CADTH, 2024).

Specific to repeat lipase testing, they do not recommend reordering lipase tests:

- "Do not reorder lipase tests for monitoring patients with an established diagnosis of acute pancreatitis.
- Do not reorder lipase tests for monitoring patients with an established diagnosis of chronic pancreatitis.
  An exception to this recommendation is if there is clinical suspicion of acute-on-chronic pancreatitis, where lipase testing is required for diagnostic purposes" (CADTH, 2024).
  Implementation advise for this recommendation: "To support reductions in unnecessary retesting, in outpatient or community settings, labs may consider implementing a 6-month hard stop minimum retesting interval.
  This recommendation is based on the experience of the advisory panel as no relevant information for serum lipase retesting for chronic pancreatitis was identified in the literature review" (CADTH, 2024).

Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations

(NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
82150	Amylase
83519	Immunoassay for analyte other than infectious agent antibody or infectious agent antigen; quantitative, by radioimmunoassay (eg, RIA)
83520	Immunoassay for analyte other than infectious agent antibody or infectious agent antigen; quantitative, not otherwise specified
83529	Interleukin-6 (IL-6)
83690	Lipase
84145	Procalcitonin (PCT)
86140	C-reactive protein

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

Evidence-based Scientific References

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Revision History

Revision Date	Summary of Changes
09/04/2024	Annual Review: Updated background, guidelines, and evidence-based scientific references. Literature review necessitated the following changes to the coverage criteria: Edited CC2 for complete clarity on the disallowance of serum lipase or amylase for individuals who have already been diagnosed with acute pancreatitis or for those who have been diagnosed with chronic pancreatitis. Now reads: "2) Measurement of serum lipase and/or amylase concentration DOES NOT MEET COVERAGE CRITERIA in any of the following situations: a) For individuals with an established diagnosis of acute or chronic pancreatitis. b) More than once per visit. c) For asymptomatic individuals during a general exam without abnormal findings." Addition of new CC6: "6) For all other situations or conditions not described above, measurement of serum lipase and/or amylase DOES NOT MEET COVERAGE CRITERIA." Updated signs and symptoms of acute pancreatitis in Note 1 based on new source material to better address all major signs and symptoms

Parathyroid Hormone, Phosphorus, Calcium, and Magnesium Testing

Policy Number: AHS – G2164 – Parathyroid Hormone, Phosphorus, Calcium, and Magnesium Testing	Prior Policy Name and Number, as applicable:
Initial Presentation Date: 03/01/2019 Revision date: 03/06/2024	

Policy Description

Parathyroid hormone (PTH), along with calcitriol and fibroblast growth factor 23 (FGF23), regulate calcium and phosphate homeostasis. PTH modulates the serum ionized calcium concentration by stimulating kidney reabsorption of calcium as well as increasing bone resorption within minutes of PTH secretion. Primary hyperparathyroidism presents itself with hypercalcemia and elevated PTH levels and is typically caused by parathyroid adenoma or hyperplasia. Secondary hyperparathyroidism is seen "in patients with kidney failure who have increased secretion of PTH [and] is related not only to gland hyperplasia and enlargement but also to reduced expression of CaSRs [calcium-sensing receptors] and, perhaps, its downstream signaling elements" (Mannstadt, 2023).

Calcium is an essential metal found in its biologically relevant divalent cation (Ca^{2+}) form in vivo. It is involved in many important biological processes, including cell signaling, signal transduction, and muscle contraction. Only 45% of the plasma calcium is in the ionized form (or 'free' form), which is the physiologically active form, while the rest is bound to albumin or complexed to anions, such as phosphate or citrate (Singh., 2023). Both total calcium and ionized calcium can be tested from a blood sample. Occasionally, calcium concentration is determined from a 24-hour urine sample (Fuleihan & Silverberg, 2023).

Phosphorus is typically used in its oxidized phosphate polyatomic ionic form (PO_4^{3-}) in vivo and is an important functional group in all classes of biomolecules—carbohydrates, proteins, lipids, and nucleic acids. The cytosol uses a phosphate-based buffer to maintain pH homeostasis. Plasma phosphorus can be in either organic or inorganic form, but the inorganic phosphates are regulated by hormones, primarily PTH. Typically, phosphate/phosphorus testing is performed on a blood sample but it can also be performed on a urine sample (Jason R Stubbs, 2024).

Magnesium, like calcium, in vivo is in its divalent cation (Mg^{2+}) form. It is involved in many enzymatic mechanisms as well as structural functions for both proteins and nucleic acids. Magnesium is required for maintenance of bone health as well as proper nerve conduction, muscle contraction, and energy production. Currently, magnesium is tested from a blood sample or less frequently from a 24-hour urine sample. Due to the large amounts of magnesium that is filtered and the degree of reabsorption and secretion in urine levels, "magnesium levels in the urine do not correlate with either the amount of magnesium ingested or the magnesium status in the body." (Workinger et al., 2018)

Related Policies

Policy Number	Policy Title
AHS-G2005	Vitamin D Testing

Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) Serum intact parathyroid (PTH) testing **MEETS COVERAGE CRITERIA** in **any** of the following situations:
   a) To assess for possible hyperparathyroidism in individuals with hypercalcemia.
   b) To assess post-operative results of parathyroid surgery.
   c) As part of annual testing of an individual previously diagnosed with hyperparathyroidism.
   d) As part of an assessment of chronic kidney disease (CKD).
   e) As part of an assessment of osteoporosis.
   f) As part of a diagnosis and/or an assessment of cancer or cancer therapy.

2) For individuals suspected of having hypoparathyroidism, pseudohypoparathyroidism, or a related disorder, serum intact parathyroid (PTH) testing (see Note 1) **MEETS COVERAGE CRITERIA** in **any** of the following situations:
   a) In the initial assessment and diagnosis of the disorders listed in Note 1.
   b) To monitor disease and/or therapy.

3) Serum intact parathyroid (PTH) testing to screen for asymptomatic hyperparathyroidism **DOES NOT MEET COVERAGE CRITERIA**.

4) For individuals presenting for a wellness visit or a general exam without abnormal findings, the following tests **DO NOT MEET COVERAGE CRITERIA**:

a) Serum, blood, or fecal magnesium testing.

b) Serum phosphorus or phosphate testing.

c) Urine phosphorus or phosphate testing.

d) Serum total calcium, serum ionized calcium, or urine calcium testing.

e) Serum parathyroid hormone testing.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

5) Testing serum for truncated parathyroid hormone metabolites (e.g., amino-terminal and carboxy-terminal fragments) **DOES NOT MEET COVERAGE CRITERIA**.

NOTES:

Note 1: Conditions of hypoparathyroidism, pseudohypoparathyroidism, and related disorders (Mantovani et al., 2018)

1. Hypoparathyroidism
2. Pseudohypoparathyroidism Type 1A (PHP1A)—due to maternal loss of function mutation at the *GNAS* coding sequence
3. Pseudohypoparathyroidism Type 1B (PHP1B)—due to methylation defect at the *GNAS* coding sequence
4. Pseudopseudohypoparathyroidism (PPHP)—due to paternal loss of function mutation at the *GNAS* coding sequence
5. Progressive Osseous Heteroplasia (POH)—due to paternal loss of function mutation at the *GNAS* coding sequence
6. Acrodysostosis (ACRDYS1)—due to mutation in *PRKAR1A*
7. Acrodysostosis (ACRDYS2)—due to mutation in *PDE4D*

Table of Terminology

Term	Definition
25[OH]D	25-hydroxy-vitamin D
AACC	American Association for Clinical Chemistry
AACE	American Association of Clinical Endocrinologists
AAES	American Association of Endocrine Surgeons
AAP	American Academy of Paediatrics
ACE	American College of Endocrinology
ACRDYS1	Acrodysostosis type 1
ACRDYS2	Acrodysostosis type 2
AGA	American Gastroenterological Association
AHA	American Heart Association
AHO	Albright hereditary osteodystrophy

ALL	Acute lymphoblastic leukemia
ALP	Alkaline phosphatase
ASA	American Society of Anesthesiologists
ASCO	American Society of Clinical Oncology
ASMBS	American Society for Metabolic & Bariatric Surgery
ATA	American Thyroid Association
ATLL	Adult T-Cell leukemia/lymphoma
AUA	American Urological Association
BRUE	Brief resolved unexplained events
BUN	Blood urea nitrogen
Ca	Calcium
Ca^{2+}	Calcium in its biologically relevant divalent cation form
CAD	Coronary artery disease
CaSRs	Calcium-sensitive receptors
CBC	Complete blood count
CCO	Cancer Care Ontario
CKD	Chronic kidney disease
CLIA '88	Clinical Laboratory Improvement Amendments of 1988
CLL	Chronic lymphocytic leukemia
CMS	Centers for Medicare and Medicaid
CRPC	Castration resistant prostate cancer
CUP	Cancer of unknown primary
CVs	Coefficients of variation
EDTA	Ethylenediaminetetraacetic acid
eGFR	Estimated glomerular filtration rate
ESCC	Esophageal squamous cell carcinomas
FDA	Food and Drug Administration
FGF	Fibroblast growth factor
FGF23	Fibroblast growth factor 23
GCTB	Giant cell tumor of bone
GD	Graves' disease
GFR	Glomerular filtration rate
GNAS	*Guanine nucleotide binding protein, alpha Stimulating activity polypeptide*
GPCRs	G-protein coupled receptors
HCHC	Hypocalciuric hypercalcemia
HIV	Human immunodeficiency virus
HPFS	Health professionals follow-up study
HPT	Hyperparathyroidism (non-specific)
ICSI	Institute for Clinical Systems Improvement
IFCC	International Federation of Clinical Chemistry
IPM	Intraoperative pseudoparathyroidism monitoring

iPTH	Intact parathyroid hormone
KDIGO	Kidney Disease Improving Global Outcomes
LDH	Lactate dehydrogenase
LDTs	Laboratory-developed tests
LFTs	Liver function tests
MEN1	Multiple endocrine neoplasia type 1
MEN2	Multiple endocrine neoplasia type 2
Mg	Magnesium
Mg^{2+}	Magnesium in its divalent cation form
MS	Multiple sclerosis
NBA	National Blood Authority
NCCMH	National Collaborating Centre for Mental Health
NCCN	National Comprehensive Cancer Network
NGC	National Guideline Clearinghouse
NGS	Next generation sequencing
NHS I	Nurses' Health Study I
NHS II	Nurses' Health Study II
NICE	National Institute for Health and Care Excellence
OMA	Obesity Medical Association
PDE4D	Phosphodiesterase 4D
PHP	Pseudoparathyroidism
PHP1A	Pseudohypoparathyroidism type 1A
PHP1B	Pseudohypoparathyroidism type 1B
pHPT	Primary hyperparathyroidism
PHPT	Primary hyperparathyroidism
PO43-	Phosphorus in its oxidized phosphate polyatomic ionic form
POH	Progressive osseous heteroplasia
PPHP	Pseudopseudohypoparathyroidism
PTH	Parathyroid hormone
PTH-rP	Parathyroid hormone-related protein
rhPTH	Recombinant human parathyroid hormone
SLL	Small lymphocytic lymphoma
SOGC	Society of Obstetricians and Gynaecologists of Canada
TLS	Tumour lysis syndrome
TOS	The Obesity Society
TSH	Thyroid stimulating hormone
UA	Urine analysis
WHO	World Health Organization

Scientific Background

Parathyroid hormone (also called parathormone or PTH) is a peptide hormone that is 84 amino acids long when first secreted by the parathyroid gland. It has a biological half-life of approximately two to four minutes before being proteolyzed into smaller fragments. These truncated fragments can comprise as much as 95% of the total circulating immunoreactive PTH. PTH is released whenever the serum ionized calcium concentration decreases as detected by the calcium-sensing receptor. Once released, PTH can increase serum calcium concentrations by increasing bone resorption as well as decreasing renal calcium excretion and increasing calcitriol production (Mannstadt, 2023). The bar graph figure below is taken from Valcour et al. (2018), and shows the predominance of the truncated fragments circulating in hemodialysis patients. These truncated PTH peptides can interfere with many serum PTH testing methods (Fuleihan & Juppner, 2024; Valcour et al., 2018).

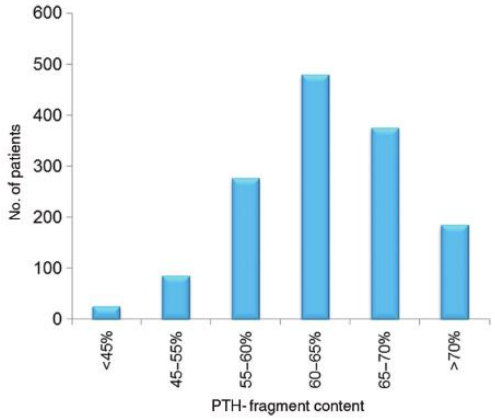

Figure 4: Frequency of fragment content in hemodialysis patients. Representative PTH fragment was assessed in 1533 hemodialysis samples by measuring both PTH(1-84) with the LIAISON method, and iPTH with Method A (high cross-reactivity with inactive fragments [4]). The fragment content was calculated as (iPTH-PTH(1-84))/iPTH and expressed as % of total iPTH.

Both PTH and PTH-related protein analogues may assist in osteoporosis therapy as each play a key role in bone metabolism; it is widely accepted that PTH is an important regulator of calcium homeostasis in the body (Wojda & Donahue, 2018). PTH has been FDA approved as an anabolic treatment for osteoporosis (Wojda & Donahue, 2018). The PTH hormone analog teriparatide is known to stimulate bone remodeling, increase the mineral density in the hip and spine bones, and reduce the risk of fractures in postmenopausal osteoporotic women (Leder, 2017). Some patients with elevated PTH levels also exhibit vitamin D deficiency, while others do not; however, elevated PTH levels seem to affect both postural stability and muscle function (Bislev et al., 2019). More research needs to be completed in this area.

Hyperparathyroidism is characterized by high serum phosphate levels, low serum calcium levels, and abnormal PTH levels; this disease is rare and can be managed with active vitamin D and calcium supplements (Marcucci et al., 2017). Researchers have noted that treatment with recombinant human parathyroid hormone (rhPTH) may be a good treatment option for patients with hyperparathyroidism who cannot maintain normal urinary and serum calcium levels (Marcucci et al., 2017).

The amount of calcium in the bloodstream is monitored by the parathyroid glands. These glands release PTH, which increases blood calcium levels. Magnesium modulates parathyroid hormone secretion;

particularly, high magnesium levels increase PTH when the parathyroid glands are exposed to low calcium levels (Rodriguez-Ortiz et al., 2014). Serum calcium may be high due to primary hyperthyroidism and malignancy, or low due to hypothyroidism or renal failure; abnormal serum calcium levels may lead to bone abnormalities or issues in the kidneys, the parathyroid gland, or the gastrointestinal tract (Shaker & Deftos, 2023).

Hypercalcemia is defined as high calcium levels in the blood stream; this may be caused by hyperparathyroidism, drugs, malignancy, or granulomatous disorders (Han et al., 2019). Hypercalcemia caused by PTH is the most common cause of primary hyperthyroidism. "Algorithms for diagnosis of PTH related hypercalcaemia require assessment of a 24-h urinary calcium and creatinine excretion to calculate calcium/creatinine clearance ratio and radiological investigations including ultrasound scan and 99mTc-sestamibi-SPECT/CT" (Han et al., 2019).

Serum phosphate homeostasis is principally regulated by the work of PTH and FGF23 via vitamin D. PTH primarily regulates calcium metabolism with secondary effects on phosphate whereas FGF23 is the opposite. Primary hyperparathyroidism (PHPT) often results in hypophosphatemia, but PTH resistance either due to surgical ablation or autoimmune disorders can cause hyperphosphatemia. PTH increases the release of phosphate from bone and the absorption of intestinal phosphate, but it increases the renal excretion of phosphate (Lederer, 2014).

Typically, serum magnesium homeostasis is regulated by the kidneys. However, large increases in PTH increases bone resorption and can also affect the loop of Henle, the location of magnesium reabsorption in the kidneys, to decrease magnesium excretion (Quamme, 1986). Certain types of tumor cells, including esophageal squamous cell carcinomas (ESCC) release a parathyroid hormone-related protein (PTH-rP). A study by Konishi et al. (2018) has demonstrated that PTH and PTH-rP affect magnesium homeostasis in ESCC receiving cisplatin therapy. The researchers found that "intravenous Mg supplementation therefore conferred protective effects against cisplatin-induced nephrotoxicity in patients with ESCC. Furthermore, increases in PTH or PTH-rP may have influenced the extent of nephrotoxicity" (Konishi et al., 2018). Hernandez-Becerra et al. (2020) found that, in rats, a calcium deficiency due to diet results in less magnesium identified in bones, including an apparent lower bone mineral density and a thinner cortical bone and trabecular bone porosity.

Analytical Validity

The International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) established a Working Group to research how pre-analytical conditions affected the measurement of PTH in blood samples (Hanon et al., 2013). This extensive review covered everything from circadian rhythms and how time of day affected clinical validity to storage conditions and seasonal changes. The research included data from 83 different studies. The authors note that the inclusion of EDTA to the sample will increase the stability to at least 72 hours for plasma samples and to 24 hours for serum samples. PTH concentrations in the summer are lower than in the winter months for patients in the Northern hemisphere, and it is noted that "PTH has a circadian rhythm characterized by a nocturnal acrophase and mid-morning nadir" (Hanon et al., 2013). The data was found to be contradictory concerning the validity of results obtained from frozen samples regardless of whether the sample was stored at -20°C or -80°C. PTH concentrations were also considerably higher in central blood as compared to peripheral blood (median values of 24.3 pmol/L versus 15.3 pmol/L, respectively). It is recommended that "blood samples for PTH measurement should be taken into tubes containing EDTA, ideally between 10:00 [a.m.] and 16:00 [p.m.], and plasma separated within 24 h of venipuncture. Plasma samples should be stored at four degrees Celsius and analysed within 72 h of venipuncture. Particular regard must be paid to the venipuncture site when

interpreting PTH concentration. Further research is required to clarify the suitability of freezing samples prior to PTH measurement" (Hanon et al., 2013).

The IFCC Working Group on PTH also investigated how to improve PTH testing, especially with regards to the need for common references and standards. "Recent increases in understanding of the complex pathophysiology of CKD [chronic kidney disease], which involves calcium, phosphate and magnesium balance, and is also influenced by vitamin D status and fibroblast growth factor (FGF)-23 production, should facilitate such improvement. Development of evidence-based recommendations about how best to use PTH is limited by considerable method-related variation in results, of up to five-fold, as well as by lack of clarity about which PTH metabolites these methods recognize. This makes it difficult to compare PTH results from different studies and to develop common reference intervals and/or decision levels for treatment" (Sturgeon et al., 2017). The graph below (taken from (Almond et al., 2012; Sturgeon et al., 2017)) compares the differences between various available PTH assays observed within a single patient specimen.

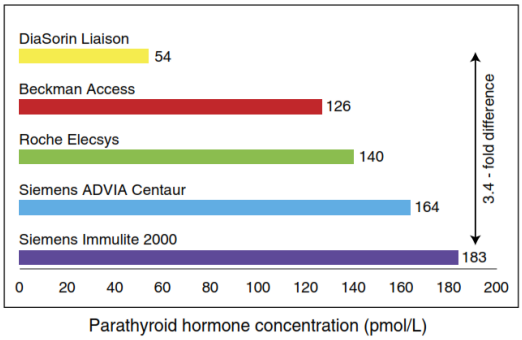

Figure 1 Between-method differences in the concentration of parathyroid hormone (PTH) observed in a typical single patient specimen

The study by Almond et al. (2012) shows that up to 4.2-fold differences can occur between these testing methods, and "these differences were sufficient to have treatment implications for 79% of the patients in the pilot study." The 2017 IFCC study shows that "within-laboratory within-method coefficients of variation (CVs) <10%"; however, "between-laboratory between-method CVs are generally >20%" (Sturgeon et al., 2017).

Bensalah et al. (2018) analyzed the differences in PTH serum measurement between the Roche Cobas e411® (which uses a chemiluminescent sandwich enzyme immunoassay) and the Abbott Architect ci8200® (which uses a chemiluminescent microparticle immunoassay); this study included 252 patients. The two techniques were compared by the Bland-Altman difference diagram. "In conclusion, our study shows a great discrepancy between the results of the PTH assay on the Architect ci8200 versus the Cobas e411," suggesting that currently marketed kits need to be evaluated further (Bensalah et al., 2018).

Clinical Utility and Validity

Since serum PTH testing can be complicated by the presence of proteolytic fragments as well as a brief biological half-life of mere minutes, Valcour et al. (2018) evaluated the efficacy of the LIAISON 1-84 PTH test, a third-generation serum test, as compared to other intact testing methods. This study was conducted at three different locations throughout the United States. Each test site recruited fifteen patients, and the patients were equally divided into three groups—healthy patients, primary

hyperparathyroid patients, and hemodialysis patients. A minimum of nine samples were collected from each patient. Each test's efficacy was also evaluated concerning how the sample was collected (plasma EDTA, unspun plasma EDTA, and serum separator) as well as how storage time at room temperature affected results (up to 72 hours). Two different standards were used—the WHO 95/646 international standard and the synthetic Bachem PTH (1-84) standard. Both the second- and third-generation intact PTH test were consistent with the standards up to 72 hours; however, the "serum is significantly less stable than plasma when samples are stored at room temperature for 72 h regardless of platform, even when separated from the clot by centrifugation within 1 h" (Valcour et al., 2018). The mean percent change from baseline ranged from 96%-107% for the LIAISON 1-84 test except for the serum at 72 h, which had a mean of 82%. Likewise, the second-generation mean percent change from baseline ranged from 95%-108% except for the serum at 72 h, which again was 82%. The authors conclude that the "LIAISON 1-84 PTH assay is accurate and reliably measures the biologically active PTH molecule in plasma or serum stored at room temperature for up [to] 27 and 24 h, respectively" (Valcour et al., 2018).

A study at the Cleveland Clinic of more than 2.7 million patients' electronic medical records was published in 2013 looking at the prevalence of PHPT, both symptomatic and asymptomatic, and the correlation with serum calcium testing. Of the records obtained, two percent had serum calcium levels >10.5 mg/dL, and 1.3% of the total patient population had previously been diagnosed with PHPT. Only 32% of the patients who had not been previously diagnosed with either hypercalcemia, PHPT, or had undergone a parathyroidectomy had recorded PTH values in their medical records. "Patients with calcium of 11.1 – 11.5 mg/dL were most likely to have PHPT (55%). Patients with calcium >12 mg/dL were most likely to have PTH measured. Of hypercalcemic patients, 67% never had PTH obtained, …. It is estimated that 43% of hypercalcemic patients are likely to have PHPT…."; The authors conclude, "it is crucial to evaluate even mild hypercalcemia, because 43% of these patients have PHPT. PHPT is underdiagnosed and undertreated" (Press et al., 2013).

In 1975, Pak and colleagues published results of a urine test they developed to diagnose hypercalciuria (Pak et al., 1975). Since then, 24-hour urinary calcium testing is a common clinical practice, especially in monitoring kidney health, with reference values of <250 mg/24 hours for males and <200 mg/24 hours for females (Mayo, 2018a). A comprehensive study by Curhan et al. (2001) investigated the 24-hour urine concentrations of calcium, magnesium, and phosphorus along with several other analytes. Calcium and magnesium were quantified by atomic absorption spectroscopy whereas phosphorus was measured using a Cobas centrifugal analyzer. Samples were collected from over 1000 patients who were already taking part in three large-scale ongoing cohort studies—NHS I (Nurses' Health Study I), NHS II, and HPFS (Health Professionals Follow-Up Study). Neither magnesium nor phosphate was significant in any of the three cohorts between the patients with kidney stones and the controls; however, the urine calcium concentration was significantly elevated ($p \leq 0.01$) in two of the three cohorts. One cohort, though, had 27% of the patients in the control group exhibiting hypercalciuria and only 33% of the experimental group exhibiting hypercalciuria. Conclusions state that "the traditional definitions of normal 24-hour urine values need to be reassessed, as a substantial proportion of controls would be defined as abnormal…" (Curhan et al., 2001).

Serum magnesium testing can be used in monitoring preeclampsia and hypermagnesemia. The reference values are age-dependent, but levels greater than nine mg/dL can be life-threatening (Mayo, 2018b). The evidence of causation or the use of serum magnesium in predicting preeclampsia have been inconclusive. A study by Kreepala et al. (2018) has proposed the use of serum total magnesium and ionized magnesium levels to develop a magnesium-based equation for screening of preeclampsia. This study involved 84 pregnant women including 20 controls. The remaining 64 had been diagnosed with preeclampsia after the 20th week of pregnancy. The authors determined that the serum ionized

magnesium levels were "significantly lower in preeclampsia group (23.95 ± 4.7% vs. 26.28 ± 2.3%, p = .04)." The equation that was developed has an "area of ROC for predictive accuracy of the model [of] 0.77 (p <.001). [The] ROC suggested that the score of 0.27 would be a threshold for screening preeclampsia with 70% sensitivity and 81% specificity." Kreepala et al. (2018) suggest "blood testing on total and ionized magnesium concentrations as well as calculation of ionized magnesium fraction in addition to routine antenatal care for better screening of the disease."

Serum magnesium levels have been identified to play a role in other disorders as well. Low serum magnesium levels have recently been associated with a greater coronary artery disease (CAD) risk Hamedanian et al. (2019); (Rooney et al., 2020). A total of 14446 participants were followed for one year in a large meta-analysis study. The researchers concluded that "low circulating Mg was associated with higher CAD risk than was higher Mg"; however, it was not determined whether magnesium concentration manipulation could assist in the prevention of coronary artery disease (Rooney et al., 2020). Mancuso et al. (2020) conducted a separate study that further validated the association between serum magnesium and CAD. They concluded that Mg2+ could be used to assess subclinical cardiovascular organ damage, including increased carotid artery intima-media thickness and left ventricular mass index in "hypertensive patients with asymptomatic subclinical vascular atherosclerotic disease and with higher cardiovascular risk." Higher serum Mg2+ concentrations could possibly be protective against progression of CAD as well.

Sri-Ganeshan et al. (2022) conducted a retrospective observational study in a single emergency department, measuring calcium (in 1426 patients), magnesium (in 1296 patients), and phosphate (in 1099 patients). Part of the study involved a clinical tool that analyzed patient electrolyte risk factors, (that is, abnormal calcium, magnesium, and phosphate levels). The "over-testing" of electrolytes in an ER setting is an area of concern, noted the authors. Researchers hypothesized they could use a decision-making tool to determine clinical factors associated with low and high levels of each electrolyte, then only test patients who met the criteria. The authors postulated that "patients without a single risk factor in the tool are unlikely to have clinically significant abnormal Ca, Mg or PO_4 levels and do not require [further] testing." After analyzing results, the authors found very high NPVs for both Ca and Mg, "If Ca and Mg had only been measured in patients with a risk factor for an abnormality, a very small proportion patients (approximately 1%) would not have been identified." However, the authors noted that the use of such a clinical decision-making tool appeared to be less robust when it came to phosphate testing (Sri-Ganeshan et al., 2022).

Guidelines and Recommendations

2016 American Association of Endocrine Surgeons (AAES)

The AAES released guidelines concerning primary hyperparathyroidism (pHPT) in 2016. With respect to laboratory testing, in Recommendation 1-1, these guidelines state, "The biochemical evaluation of suspected pHPT should include serum total calcium, PTH, creatinine, and 25-hydroxyvitamin D levels (strong recommendation; moderate-quality evidence)." The AAES also addresses differentiating between pHPT and suspected "familial hypocalciuric hypercalcemia, which is an autosomal dominant disorder of the renal calcium-sensing receptor that can mimic pHPT." In Recommendation 1-2 (strong recommendation; moderate-quality evidence), "a 24-hour urine measurement of calcium and creatinine should be considered in patients undergoing evaluation for possible pHPT.... Familial hypocalciuric hypercalcemia should be considered in patients with long-standing hypercalcemia, urinary calcium levels less than 10 mg/24 hours, and a calcium to creatinine clearance ratio less than 0.01." The AAES also address the use of intraoperative PTH monitoring (IPM). Recommendation 6-1: "When image-guided

focused parathyroidectomy is planned, IPM is suggested to avoid higher operative failure rates (strong recommendation; moderate-quality evidence)." However, a strong recommendation with low-quality evidence to recommendation 6-2 was provided: "Surgeons who use IPM should use a sampling protocol that is reliable in the local environment and should be familiar with the interpretation of PTH decay dynamics." The frequency of testing either calcium or PTH post-operatively is not given, but the AAES mentions these recommendations in several comments concerning the monitoring or measuring calcium and/or PTH levels or determining post-operative hyper-/hypoparathyroidism (Recommendation 14-7, Recommendation 15-1a, Recommendation 15-1b, Recommendation 15-3, Recommendation 15-4, and Recommendation 16-2). It is also stated that the definition of a success versus failure of operation is when levels are compared six months post-operation (Wilhelm et al., 2016).

2018 First International Consensus Statement on Pseudohypoparathyroidism and Related Disorders

An international consortium of representatives from across Europe and North America released their first international consensus statement, including extensive guidelines and recommendations, concerning pseudohypoparathyroidism and related disorders in 2018. These disorders have a wide array of phenotypes but are due to impaired cell signaling cascades of G-protein coupled receptors (GPCRs). Pseudohypoparathyroidism can be classified as either type 1A or 1B (PHP1A and PHP1B, respectively), depending on the type of defect in the *GNAS* coding sequence. Pseudopseudohypoparathyroidism (PPHP) and progressive osseous heteroplasia (POH) are caused by a paternal loss of function defect to *GNAS*. Acrodysostosis is classified as either type one (ACRDYS1) or type two (ACRDYS2) due to mutations in either *PRKAR1A* or *PDE4D*, respectively. PTH resistance can be negligible in infancy but typically increases with age.

In recommendation 1.3 (A+++), the guidelines list the clinical and biochemical major criteria for diagnosing PHP and related disorders, including "PTH resistance, and/or subcutaneous ossifications that can include deeper ossifications, and/or early-onset (before two years of age) obesity associated with TSH resistance or with one of the above, and/or AHO [Albright hereditary osteodystrophy] alone" regardless of family history. In recommendation 1.6 (A+++), "The definition of PTH resistance is as follows: [1] The association of hypocalcaemia, hyperphosphataemia and elevated serum levels of PTH in the absence of vitamin D deficiency and when magnesium levels and renal function are normal. [2] PTH resistance in the context of PHP and related disorders should be suspected when PTH is at, or above, the upper limit of normal, in the presence of normal calcifediol levels and elevated serum levels of phosphorus, even in the absence of overt hypocalcaemia. PTH resistance and consequent changes in serum levels of calcium, phosphorus and PTH can be variable, and repeated testing might be required." In all cases, genetic counseling is recommended.

In recommendation 3.2, the measurement of serum PTH, calcium, phosphorus, and calcifediol are recommended; moreover, "measurement of PTH, calcium and phosphorus should be performed regularly (every six months in children and at least yearly in adults) with the exception of patients carrying either a *GNAS* mutation on the paternal allele or a *PDE4D* mutation in whom, apart from diagnosis, routine assessment is not necessary. Monitoring of serum levels of calcium should be more frequent in symptomatic individuals, during acute phases of growth, during acute illness and during pregnancy and breastfeeding…." For patients undergoing vitamin D therapy, they stress as part of recommendation 3.4 (A++) that serum phosphate be monitored. Concerning patients undergoing treatment for PTH resistance, in recommendation 3.5 (A++), the guidelines state that "levels of PTH,

calcium and phosphorus should be monitored every six months in asymptomatic patients and more frequently when clinically indicated." In recommendation 3.26 (A+), the routine measurement of calcitonin is not recommended. (Mantovani et al., 2018)

2020 European Network on Pseudohypoparathyroidism (EuroPHPnet)

The EuroPHPnet published its "Recommendations for Diagnosis and Treatment of Pseudohypoparathyroidism and Related Disorders: An Updated Practice Tool for Physicians and Patients." In these guidelines, the EuroPHPnet noted that "PTH resistance is the hallmark of PHP [pseudohypoparathyroidism], found in 45-80% of patients," and symptoms of PTH resistance should not be ignored and "screening and follow-up of PTH resistance should include measurement of PTH, 25-OH vitamin D, calcium, and phosphate every three to six months in children and at least yearly in adults." However, the frequency of monitoring is also contingent on whether the individual is symptomatic or not, in acute phases of growth, experiencing intercurrent illness, pregnancy, or is breastfeeding. In the case of pregnant women with hypocalcemia and/or hypothyroidism, they "should be monitored following the international guidelines for any pregnancy associated with these disturbances" and their newborns "should be evaluated for the presence of skin ossifications and levels of TSH, calcium, and phosphorous" (Mantovani et al., 2020).

International Workshop on the Evaluation and Management of Primary Hyperparathyroidism

The Fifth International Workshop on the Evaluation and Management of Primary Hyperparathyroidism convened in 2022 and published their guidelines as a consensus statement in *The Journal of Bone and Mineral Research*.

For the diagnosis of asymptomatic hypercalcemic PHPT "where biochemical screening is commonly performed, most patients with PHPT come to clinical attention when hypercalcemia is found unexpectedly in the context of an investigation of an unrelated problem or simply upon routine testing. If the PTH level is also found to be high, or even in the normal range, the most likely diagnosis is asymptomatic hypercalcemic PHPT."

When diagnosing normocalcemic PHPT, "PTH levels may be measured in the evaluation of medical conditions such as osteoporosis, low bone mass, or nephrolithiasis. Normocalcemic PHPT (NPHPT) is characterized by persistently normal albumin-adjusted total and ionized serum calcium levels, accompanied by elevated levels of PTH on at least two consecutive measurements, over a three month to six month period" (Bilezikian et al., 2022).

The workshop also included a section on genetic testing where they note that testing for mutations in one of 10 genes can facilitate the diagnosis of a syndromic or nonsyndromic form of PHPT, which helps in clinical management and treatment. Specifically, they note that "genetic testing helps to identify family members who may or may not be at risk. Genetic counseling and evaluation, thus, should be considered for patients < 30 years with PHPT, those with multigland disease by history or imaging, those with a family history of hypercalcemia or syndromic diseases such as MEN1, MEN2A, MEN4, or HPT-JT syndrome, and in patients with atypical parathyroid adenoma and parathyroid carcinoma" (Bilezikian et al., 2022).

The 2014 workshop established guidelines for monitoring patients with asymptomatic primary hyperparathyroidism (PHPT) and recommended annual testing of serum calcium. A formula was given to determine corrected calcium concentration, which is recommended (rather than using free calcium),

since "most centers do not have sufficient capabilities to rely upon an ionized, free calcium concentration":

Corrected [Ca] = [total serum calcium in mg/dL + 0.8*(4.0 - patient's serum albumin in g/dL)]

Recommendations for evaluating asymptomatic PHPT are shown in Table 3 shown below although the guidelines do state that "this evaluation is for PHPT, not to distinguish between PHPT and other causes of hypercalcemia." This table includes calcium (both serum and 24-hour urine testing) and phosphate testing.

Table 3. Recommendations for the Evaluation of Patients With Asymptomatic PHPT

Recommended
Biochemistry panel (calcium, phosphate, alkaline phosphatase activity, BUN, creatinine), 25(OH)D
PTH by second- or third-generation immunoassay
BMD by DXA
Lumbar spine, hip, and distal 1/3 radius
Vertebral spine assessment
X-ray or VFA by DXA
24-h urine for:
Calcium, creatinine, creatinine clearance
Stone risk profile
Abdominal imaging by x-ray, ultrasound, or CT scan
Optional
HRpQCT
TBS by DXA
Bone turnover markers (bone-specific alkaline phosphatase activity, osteocalcin, P1NP [select one]; serum CTX, urinary NTX [select one])
Fractional excretion of calcium on timed urine sample
DNA testing if genetic basis for PHPT is suspected

Abbreviations: BUN, blood urea nitrogen; P1NP, procollagen type 1 N-propeptide; CTX, C-telopeptide cross-linked collagen type I; NTX, N-telopeptide of type I collagen. This evaluation is for PHPT, not to distinguish between PHPT and other causes of hypercalcemia.

In their algorithm for monitoring patients with normocalcemic PHPT, both annual calcium and PTH testing are included; however, there is no mention of the method of calcium testing (i.e. serum versus 24-hour urine testing) or phosphate testing (Bilezikian et al., 2014).

National Comprehensive Cancer Network (NCCN)

The NCCN addresses PTH, calcium, phosphate, and magnesium testing in several different guidelines.

Neuroendocrine & Adrenal Tumors (NCCN, 2023c): The NCCN continues to assert that "Primary hyperparathyroidism associated with parathyroid adenomas is the most common manifestation of MEN1 [Multiple endocrine neoplasia, type 1]. Measurement of serum calcium levels is recommended if hyperparathyroidism is suspected. If calcium levels are elevated, parathyroid hormone (PTH) and 25-OH vitamin D levels should be checked." With respect to the surveillance of MEN1-associated parathyroid tumors, "The panel recommends annual calcium and serum PTH levels to screen for parathyroid tumors. If calcium levels rise, 25-OH vitamin D should be measured and imaging with neck ultrasound and/or parathyroid sestamibi with SPECT scan (SPECT-CT preferred) or 4D-CT should be performed." Similarly, for the evaluation of patients with Multiple Endocrine Neoplasia Type 2 (MEN2), "serum calcium levels should be measured. If it is found to be elevated, PTH and 25-OH vitamin D levels should be measured. A neck ultrasound, sestamibi scan with SPECT, or 4D-CT scan can also be performed as appropriate."

Acute Lymphoblastic Leukemia (ALL) (NCCN, 2023a): As part of the initial workup for ALL patients, they recommend "a tumor lysis syndrome (TLS) panel (including measurements for serum lactate dehydrogenase [LDH], uric acid, potassium, phosphates, and calcium)." In the section concerning the supportive care of ALL in steroid management, they guidelines state to "obtain vitamin D and calcium status and replete as needed" and monitor possible osteonecrosis/avascular necrosis associated as a potential long-term side effect of corticosteroids. Likewise, the NCCN later stated "To monitor patients for risks of developing symptomatic osteonecrosis, routine measurements for vitamin D and calcium levels should be obtained and periodic radiographic evaluation (using plain films or MRI [magnetic resonance imaging]) should be considered."

Systemic Light Chain Amyloidosis (NCCN, 2024g): As part of the initial diagnostic workup, in the section titled "Laboratory evaluation (directed toward commonly affected organ systems)," the NCCN recommends testing "serum BUN [blood urea nitrogen]/creatinine, electrolytes, albumin, calcium, serum uric acid, serum LDH, and beta-2 microglobulin."

Bone Cancer (NCCN, 2024a): In the section concerning the workup of Giant Cell Tumor of Bone (GCTB), a rare benign tumor, the guidelines state that "brown tumor of hyperparathyroidism should be considered as a differential diagnosis; routine evaluation of serum calcium, phosphate, and parathyroid hormone levels can help exclude this diagnosis." Moreover, prior to treatment of bone lesions, it is recommended that "Laboratory studies, such as complete blood count (CBC), comprehensive metabolic panel (CMP) with calcium to assess for hypercalcemia, lactate dehydrogenase (LDH), and alkaline phosphatase (ALP) should be done prior to initiation of treatment."

Breast Cancer (NCCN, 2024b): In general, in monitoring metastatic disease, "laboratory tests such as alkaline phosphatase, liver function, blood counts, and calcium…" are to be included to help aid the clinician in determining "the effectiveness of treatment and the acceptability of toxicity."

Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma (CLL/SLL) (NCCN, 2024c): Small-molecule inhibitors, such as Venetoclax, are possible therapies for CLL/SLL. Tumor Lysis Syndrome (TLS) is a possible side effect of such treatment. In the section on supportive care for CLL/SLL, they note that "patients with bulky lymph nodes, progressive disease after small-molecular inhibitor therapy, and receiving chemotherapy, venetoclax, lenalidomide, obintuzumab are considered to be at high-risk for TLS." NCCN further states that laboratory hallmarks of TLS include high potassium, uric acid, phosphorous, lactate dehydrogenase, and low calcium. In Venetoclax therapy, particularly, they state to "evaluate blood chemistries (potassium, uric acid, phosphorus, calcium, and creatinine); review in real time." The table below (adapted from the guideline) depicts the blood chemistry monitoring as recommended:

Blood Chemistry Monitoring (potassium uric acid, phosphorus, calcium and creatinine)	
Low Tumor Burden	
Outpatient setting	Pre-dose, 6-8 hours, 24 hours at first dose of 20mg and 50mg Pre-dose at subsequent ramp-up doses
Medium Tumor Burden	
Outpatient setting	Pre-dose, 6-8 hours, 24 hours at first dose of 20mg and 50mg Pre-dose at subsequent ramp-up doses

	Consider hospitalization for patients with CrCl <80 mL/min at first dose of 20 mg and 50 mg
High Tumor Burden	
In hospital setting	At first dose of 20 and 50 mg Pre-dose 4 hrs 8 hrs 12 hrs 24 hrs
Outpatient setting (for subsequent ramp-up doses)	Pre-dose 6-8 hrs 24 hrs

Esophageal and Esophagogastric Junction Cancers (NCCN, 2023b): In the section on principles of survivorship under *Management of Long-Term Sequelae of Disease or Treatment*, they say to "consider monitoring vitamin B, folic acid, vitamin D, and calcium levels." Moreover, following esophagectomy, long-term calcium deficiency is common along with deficiencies in vitamin B_{12}, folic acid, and vitamin D.

Kidney Cancer (NCCN, 2024d): The NCCN uses serum calcium levels as a predictor of short survival used to select patients for temsirolimus, as well as a prognostic factor [i.e. "calcium > upper limit of normal (Normal: 8.5-10.2 mg/dL)"] in accordance with the Memorial Sloan Kettering Cancer Center Prognostic Model and the International Metastatic Renal Cell Carcinoma Database Consortium Criteria. The guidelines do not state how frequently serum calcium should be tested or if it is solely for use at diagnosis. However, the guidelines recommend that laboratory evaluation for patients with renal cell carcinoma typically present with a suspicious mass involving the kidney may include a metabolic panel consisting of "corrected calcium, serum creatinine, liver function studies, and urinalysis."

Multiple Myeloma (NCCN, 2024e): In the initial diagnostic workup for multiple myeloma, the NCCN recommends testing "serum BUN/creatinine, electrolytes, liver function tests, albumin, calcium, serum uric acid, serum LDH, and beta-2 microglobulin." As follow-up to the clinical presentation of either "solitary plasmacytoma" (with minimal marrow involvement or less) or "smoldering (asymptomatic)" myeloma, again "corrected calcium" is listed as one of the recommended blood tests. Calcium is also recommended following treatment of active myeloma, and an elevated calcium concentration is listed as one of the "direct indicators of increasing disease and/or end organ dysfunction" since "excess bone resorption from bone disease can lead to excessive release of calcium into the blood, contributing to hypercalcemia."

Occult Primary (Cancer of Unknown Primary [CUP]) (NCCN, 2024f): "Routine laboratory tests (ie, complete blood count [CBC], electrolytes, liver function tests [LFTs], creatinine, calcium), occult blood stool testing, and contrast-enhanced chest/abdominal/pelvic CT scans with IV contrast are also recommended" for patients with a suspected metastatic malignancy.

Prostate Cancer (NCCN, 2023d): In the section concerning the treatment with denosumab, the guidelines state that "hypocalcemia is seen twice as often with denosumab than zoledronic acid and all patients on denosumab should be treated with vitamin D and calcium with periodic monitoring of serum calcium levels." In the section concerning patients with castration resistant prostate cancer (CRPC), the NCCN states, "hypocalcemia should be corrected before starting denosumab, and serum calcium monitoring is

required for denosumab and recommended for zoledronic acid, with repletion as needed." In treatment of CRPC with abiraterone acetate, "monitoring of liver function, potassium and phosphate levels, and blood pressure readings on a monthly basis is warranted during abiraterone therapy." Men with CRPC are at a higher risk for severe hypocalcemia and hypophosphatemia due to use of denosumab.

T-Cell Lymphomas (NCCN, 2024h): For adult T-Cell Leukemia/Lymphoma (ATLL), the NCCN states, "the initial workup for ATLL should include a complete history and physical examination…a CBC with differential and complete metabolic panel (serum electrolyte levels, calcium, creatinine, and blood urea nitrogen) and measurement of serum LDH levels." Under the supportive care section for T-Cell lymphomas, the NCCN recommends monitoring for tumor lysis syndrome (TLS), which include measuring serum phosphorous and calcium levels since "laboratory TLS is defined as a 25% increase in the levels of serum uric acid, potassium, or phosphorus or a 25% decrease in calcium levels."

Thyroid Carcinoma (NCCN, 2024i): In the algorithm for thyroid carcinoma-medullary carcinoma, both serum calcium and PTH are recommended as additional workup for patients who have MEN2A/Familial medullary thyroid carcinoma (codon 609, 611, 618, 620, 630, 634, 768, 790, 791, 804, or 891 *RET* mutations). Serum calcium testing is among the testing and procedures recommended upon diagnosis of medullary thyroid carcinoma.

2012, 2017 Kidney Disease Improving Global Outcomes (KDIGO)

Kidney Disease Improving Global Outcomes released their *Clinical practice guideline for the Evaluation and Management of Chronic Kidney Disease* (CKD) in 2012 and then their *Clinical Practice Guideline Update for the Diagnosis, Evaluation, Prevention, and Treatment of Chronic Kidney Disease-Mineral and Bone Disorder (CKD-MBD)* in 2017. In the 2012 guidelines (KDIGO, 2013), in recommendation 3.3.1 (1C), they state, "We recommend measuring serum levels of calcium, phosphate, PTH, and alkaline phosphatase activity at least once in adults with GFR [glomerular filtration rate] <45 ml/min/1.73 m^2 (GFR categories G3b-G5) in order to determine baseline values and inform prediction equations if used." In recommendation 3.3.4 (2C recommendation strength), for people in GFR categories G3b-G5 they "suggest that people with levels of intact PTH above the upper normal limit of the assay are first evaluated for hyperphosphatemia, hypocalcemia, and vitamin D deficiency." With regards to serum phosphate levels, they recommend that they are maintained "in the normal range according to local laboratory reference values" (recommendation 3.3.3; 2C). The guidelines, however, do not state a recommendation with respect to the frequency of testing past initial baseline and do not address magnesium testing other than to list renal magnesium wasting as a criterion for CKD.

The 2017 guidelines (KDIGO, 2017) in recommendation 3.1.1 state: "We recommend monitoring serum levels of calcium, phosphate, PTH, and alkaline phosphatase activity beginning in CKD G3a (*1C*). In children, we suggest such monitoring beginning in CKD G2 (*2D*)." Recommendation 3.1.2 (*Not graded*) addresses the frequency of such testing and says, "to base the frequency…on the presence and magnitude of abnormalities, and the rate of progression of CKD." The table below lists the "reasonable monitoring intervals":

CKD Stage	Test	Reasonable Monitoring Interval
G3a-G3b	Serum Calcium	Every 6-12 months
G3a-G3b	Serum Phosphate	Every 6-12 months

G3a-G3b	PTH	"Based on baseline level and CKD progression"
G4	Serum Calcium	Every 3-6 months
G4	Serum Phosphate	Every 3-6 months
G4	PTH	Every 6-12 months
G5	Serum Calcium	Every 1-3 months
G5	Serum Phosphate	Every 1-3 months
G5	PTH	Every 3-6 months
G4-G5D	Alkaline Phosphatase Activity	Every 12 months, or more frequently in the presence of elevated PTH

Recommendation 3.2.3 (*2B*) suggests measuring either PTH or bone-specific alkaline phosphatase to assess bone disease. For patients with CKD G3a-G5D, their treatment "should be based on serial assessments of phosphate, calcium, and PTH levels, considered together" (Recommendation 4.1.1 *Not Graded*). Recommendation 4.2.1 (*2C*) states: "In patients with CKD G3a-G5 not on dialysis, the optimal PTH level is not known. However, we suggest that patients with levels of intact PTH progressively rising or persistently above the upper normal limit for the assay be evaluated for modifiable factors, including hyperphosphatemia, hypocalcemia, high phosphate intake, and vitamin D deficiency." Recommendation 5.2 (*Not Graded*) addressed the frequency of testing post-kidney transplant. The table below contains the information regarding the reasonable monitoring intervals:

CKD Stage	Test	Reasonable Monitoring Interval
G1T-G3bT	Serum Calcium	Every 6-12 months
G1T-G3bT	Serum Phosphate	Every 6-12 months
G1T-G3bT	PTH	Once, with subsequent intervals depending on baseline level and CKD progression
G4T	Serum Calcium	Every 3-6 months
G4T	Serum Phosphate	Every 3-6 months
G4T	PTH	Every 6-12 months
G5T	Serum Calcium	Every 1-3 months
G5T	Serum Phosphate	Every 1-3 months
G5T	PTH	Every 3-6 months

G3aT-G5T	Alkaline Phosphatase Activity	Annually, or more frequently in the presence of elevated PTH

Within recommendation 5.6 (*2C*), KDIGO recommends "treatment choices be influenced by the presence of CKD-MBD, as indicated by abnormal levels of calcium, phosphate, PTH, alkaline phosphatases, and 25(OH)D" (KDIGO, 2017).

American Urological Association (AUA)

In 2013, the AUA published *Follow-up for Clinically Localized Renal Neoplasms*. In recommendation two, as an *Expert Opinion*, the AUA states, "Patients undergoing follow-up for treated or observed renal masses should undergo basic laboratory testing to include blood urea nitrogen (BUN)/creatinine, urine analysis (UA) and estimated glomerular filtration rate (eGFR). Other laboratory evaluations, including complete blood count (CBC), lactate dehydrogenase (LDH), liver function tests (LFTs), alkaline phosphatase (ALP) and calcium level, may be used at the discretion of the clinician."

The AUA published their guidelines titled *Medical Management of Kidney Stones* in 2014. These guidelines were reviewed, and validity was confirmed in 2019 (Pearle et al., 2019). In recommendation two, the AUA recommends that "clinicians should obtain serum intact parathyroid hormone (PTH) level as part of the screening evaluation if primary hyperparathyroidism is suspected." Also recommend (Recommendations six and seven) is that "metabolic testing should consist of one or two 24-hour urine collections obtained on a random diet and analyzed at minimum for total volume, pH, calcium, oxalate, uric acid, citrate, sodium, potassium and creatinine" but that "clinicians should not routinely perform 'fast and calcium load' testing to distinguish among types of hypercalciuria" (Pearle et al., 2019).

2014-2021 National Institute for Health and Care Excellence (NICE)

NICE, like the NCCN, addresses PTH, calcium, phosphate, and magnesium testing in several different guidelines.

2014 Bipolar disorder: assessment and management (NCCMH, 2023): In recommendation 1.2.12, they recommend annual calcium screening for anyone on a long-term lithium therapy regimen; however, in recommendation 1.10.21, they recommend testing "for urea and electrolytes including calcium…every six months, and more often if there is evidence of impaired renal or thyroid function, raised calcium levels or an increase in mood symptoms that might be related to impaired thyroid function." In recommendation 1.10.14, when a patient begins a lithium regimen, a clinician should test "for urea and electrolytes including calcium, estimated glomerular filtration rate (eGFR), thyroid function and a full blood count."

2014 Multiple sclerosis in adults: management (NICE, 2022): In recommendation 1.1.4, they recommend calcium testing along with full blood count, inflammatory markers, liver and renal function tests, glucose, thyroid function tests, vitamin B_{12}, and HIV [human immunodeficiency virus] serology testing "before referring a person suspected of having MS to a neurologist" to "exclude alternative diagnoses."

2015 Suspected cancer: recognition and referral (NICE, 2023): In the section concerning myeloma, in recommendation 1.10.4, they state, "offer a full blood count, blood tests for calcium and plasma viscosity or erythrocyte sedimentation rate to assess for myeloma in people aged 60 and over with persistent bone pain, particularly back pain, or unexplained fracture."

2019 Clinical practice guideline: undernutrition in chronic kidney disease (Wright et al., 2019): These guidelines include a section regarding the nutritional status of an individual with chronic kidney disease. The NICE states that "Assessment of nutritional status should therefore be considered when patients begin education for kidney replacement treatment as part of their overall care as well as for potential intervention regarding salt, potassium, phosphate and protein / energy intake assessments" (Wright et al., 2019). Specific assessment methods are not mentioned.

2021 Chronic kidney disease: assessment and management (NICE, 2021): In recommendation 1.11.9, within the section concerning the use of phosphate binders for children and young people, they state to "offer children and young people with CKD stage 4 or 5 and hyperphosphataemia a calcium-based phosphate binder to control serum phosphate levels." In the continuation via recommendation 1.11.10, they also state, "if serum calcium increases towards, or above, the age-adjusted upper normal limit: ●investigate possible causes other than the phosphate binder ●consider reducing the dose of the calcium-based phosphate binder and adding sevelamer carbonate or switching to sevelamer carbonate alone [2021]." When discussing phosphate binders for adults, they state in their recommendation 1.11.12 for the first phosphate binder, "offer adults with CKD stage 4 or 5 and hyperphosphataemia calcium acetate to control serum phosphate levels [2021]." If calcium acetate is not indicated, "for example, because of hypercalcaemia or low serum parathyroid hormone levels," or not tolerated, recommendation 1.11.13 states to offer sevelamer carbonate. Recommendations 1.11.14 and 1.11.15 continue by offering sucroferric oxyhydroxide, if an adult is on dialysis and a calcium-based phosphate binder is not needed; calcium carbonate, "if a calcium-based phosphate binder is needed"; and lanthanum carbonate "for adults with CKD stage 4 or 5 if other phosphate binders cannot be used." In the 2021 update, they also state in recommendation 1.11.18, "at every routine clinical review, assess the person's serum phosphate control, taking into account: ●diet ●whether they are taking the phosphate binders as prescribed ●other relevant factors, such as vitamin D levels, serum parathyroid hormone levels, alkaline phosphatase, serum calcium, medications that might affect serum phosphate, or dialysis [2021]." These guidelines mention serum phosphate, serum calcium, and PTH; however, they do not state when these tests should be performed or the frequency of testing.

In recommendation 1.12.1, they do not recommend to "routinely measure calcium, phosphate, parathyroid hormone (PTH) and vitamin D levels in people with a GFR of 30 ml/min/1.73 m^2 or more (GFR category G1, G2, or G3)." Then, in the following recommendation, they do recommend measuring serum calcium, PTH, and phosphate for patients in GFR categories G4 or G5. "Determine the subsequent frequency of testing by the measured values and the clinical circumstances. If doubt exists, seek specialist opinion." They recommend in 1.12.7 to "monitor serum calcium and phosphate concentrations in people receiving alfacalcidol or calcitriol supplements."

2021 American Society of Clinical Oncology (ASCO)/Cancer Care Ontario (CCO)

The CCO and ASCO convened a working group in 2017 concerning the use of bisphosphonates in breast cancer and published their recommendations in the *Journal of Clinical Oncology*. They clearly state that "patients should have serum calcium measured prior to starting treatment. Patients receiving intravenous bisphosphonates (zoledronic acid) should be monitored for renal function prior to starting this treatment, and for serum calcium and increase in serum creatinine throughout the treatment period."

A 2021 update from the CCO and ASCO group reaffirmed the statement above (Eisen et al., 2022).

American Association of Clinical Endocrinologists (AACE)/American College of Endocrinology (ACE)

In 2020, the AACE/ACE updated its 2016 guidelines concerning osteoporosis in post-menopausal women, now recommending "a complete blood count, comprehensive metabolic panel, 25-hydroxyvitamin D (25[OH]D), intact parathyroid hormone (PTH), phosphate, and a 24-hour urine collection for calcium, sodium, and creatinine" in evaluating osteoporosis. The guidelines note that "the 24-hour urine calcium collection must occur after the patient is replete of vitamin D and has been on a reasonable calcium intake (1,000-1,200 mg/day) for at least 2 weeks. If the patient is receiving thyroid hormone or there is suspicion for hyperthyroidism, thyroid-stimulating hormone should also be obtained" (Camacho et al., 2020).

In the 2017 guidelines for the management of dyslipidemia prevention of cardiovascular disease, the AACE/ACE highlighted the use of coronary artery calcium scores in the detection of cardiovascular risk, stating that coronary artery calcium scoring "is recognized by the AHA [American Heart Association] as a surrogate marker for coronary heart disease" (Jellinger et al., 2017).

2014 and 2022 Society of Obstetricians and Gynaecologists of Canada (SOGC)

The 2014 SOGC guidelines concerning hypertensive disorders during pregnancy recommend using magnesium supplements for pregnant women; however, the SOGC clearly states in recommendation #120 that "routine monitoring of serum magnesium levels is not recommended" (Magee et al., 2014).

However, in the updated 2022 guidelines there is no mention of magnesium testing, only a recommendation for magnesium sulphate as a first-line treatment of eclampsia and prophylaxis against eclampsia in women with preeclampsia and severe hypertension or adverse maternal conditions (strong, high) (Magee et al., 2022).

2022 American Heart Association (AHA) /American College of Cardiology (ACC) /Heart Failure Society of America (HSFA) Guideline for the Management of Heart Failure

The 2022 guideline concerning heart failure mentions both magnesium and calcium testing for patients with heart failure (HF), "Laboratory evaluation with complete blood count, urinalysis, serum electrolytes (including sodium, potassium, calcium, and magnesium), blood urea nitrogen, serum creatinine, glucose, fasting lipid profile, liver function tests, iron studies (serum iron, ferritin, transferrin saturation), and thyroid-stimulating hormone level and electrocardiography is part of the standard diagnostic evaluation of a patient with HF" (Heidenreich et al., 2022).

2016 American Academy of Pediatrics (AAP)

The AAP in 2016 issued guidelines concerning brief resolved unexplained events (BRUE) in infants. "The term BRUE is defined as an event occurring in an infant younger than 1 year when the observer reports a sudden, brief, and now resolved episode of ≥1 of the following: (1) cyanosis or pallor; (2) absent, decreased, or irregular breathing; (3) marked change in tone (hyper- or hypotonia); and (4) altered level of responsiveness." For infants between 60 days and <1 year in age, in recommendation 6B under IEM (inborn error of metabolism), the AAP states that "clinicians should not obtain a measurement of serum sodium, potassium, chloride, blood urea nitrogen, creatinine, calcium, or ammonia to detect an IEM on infants presenting with a lower-risk BRUE (Grade C, Moderate Recommendation)" (Tieder et al., 2016).

2013 American Association of Clinical Endocrinologists (AACE)/American College of Endocrinology (ACE)/The Obesity Society (TOS)

The joint task force between AACE, ACE, and TOS issued *Clinical Practice Guidelines for Healthy Eating for the Prevention and Treatment of Metabolic and Endocrine Diseases in Adults* in 2013. With regards to CKD in recommendation R29, they state, "If the intact parathyroid hormone (PTH) level remains elevated above treatment goal despite a serum 25(OH)D level higher than 30 ng/mL, treatment with an active form of vitamin D is indicated (Grade A, BEL 1)." As part of recommendation R32, they state, "A 24-hour urine calcium collection should be measured in patients with osteoporosis or patients at risk for bone loss in order to check calcium adequacy and test for hypercalciuria or malabsorption (Grade B, BEL 2)." Furthermore, "during vitamin D therapy, serum calcium and phosphorus levels need to be monitored closely to prevent hypercalcemia and hyperphosphatemia, aiming for calcium and phosphorus levels of <10.2 mg/dL and <4.6 mg/dL, respectively" (Gonzalez-Campoy et al., 2013).

2013, 2019 AACE/TOS/ASMBS (American Society for Metabolic & Bariatric Surgery)/OMA (Obesity Medical Association)/ASA (American Society of Anesthesiologists)

Also, in 2013, the AACE/TOS/ASMBS/OMA/ASA issued guidelines concerning perioperative, nonsurgical support for the bariatric surgery patient. Within recommendation R48, they state, "Bisphosphonates may be considered in bariatric surgery patients with osteoporosis only after appropriate therapy for calcium and vitamin D insufficiency…. Evaluation should include serum parathyroid hormone (PTH), total calcium, phosphorus, 25-hydroxyvitamin D, and 24-hour urine calcium levels (Grade C; BEL 3)."

The updated guidelines for the perioperative nutrition, metabolic, and nonsurgical support of patients undergoing bariatric procedures were published by the ACE, TOS, ASMBS as well as the Obesity Medicine Association, and the American Society of Anesthesiologists Boards of Directors. The guidelines give the following recommendations:

- "Patients who become pregnant following bariatric procedure should have nutritional surveillance and laboratory screening for nutrient deficiencies every trimester, including iron, folate, vitamin B12, vitamin D, and calcium, and if after a malabsorptive procedure, fat-soluble vitamins, zinc, and copper (Grade D)
- Evaluation of patients for bone loss after bariatric procedures may include serum parathyroid hormone, total calcium, phosphorus, 25-hydroxyvitamin D, and 24-hour urine calcium levels (Grade C; BEL 3)" (Mechanick et al., 2019).

2013 American Gastroenterological Association (AGA)

The 2013 AGA guidelines concerning constipation states that "although metabolic tests (thyroid-stimulating hormone, serum glucose, creatinine, and calcium) are often performed, their diagnostic utility and cost-effectiveness have not been rigorously evaluated and are probably low." Under the section *What Tests Should Be Performed to Assess for Medical Causes of Constipation?*, they state, "In the absence of other symptoms and signs, only a complete blood cell count is necessary (strong recommendation, low-quality evidence). Unless other clinical features warrant otherwise, metabolic tests (glucose, calcium, sensitive thyroid-stimulating hormone) are not recommended for chronic constipation (strong recommendation, moderate-quality evidence)."

American Thyroid Association (ATA)

The ATA has published guidelines for the diagnosis and management of hyperthyroidism and other causes of thyrotoxicosis. The ATA has stated that after a thyroidectomy, "serum calcium with or without intact parathyroid hormone (iPTH) levels can be measured,"; further, after a thyroidectomy for TMNG (toxic multinodular goiter), "serum calcium with or without iPTH levels should be measured" (Ross et al., 2016). When preparing patients with GD (Graves' disease) for a thyroidectomy, the ATA recommends that "Calcium and 25-hydroxy vitamin D should be assessed preoperatively and repleted if necessary" (Ross et al., 2016).

The ATA also published a statement regarding postoperative hypoparathyroidism. In it, they recommend to "Either treat at-risk patients empirically with calcium, or measure calcium and/or PTH in the immediate postoperative period and treat according to evidence-based protocols." (Orloff et al., 2018)

Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: http://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
82310	Calcium; total
82330	Calcium; ionized
82340	Calcium; urine quantitative, timed specimen
83735	Magnesium
83970	Parathormone (parathyroid hormone)
84100	Phosphorus inorganic (phosphate)
84105	Phosphorus inorganic (phosphate); urine

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

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Revision History

Revision Date	Summary of Changes
03/06/2024	Reviewed and Updated: Updated the background, guidelines and recommendations, and evidence-based scientific references. Literature review did not necessitate any modifications to coverage criteria.
03/01/2023	Reviewed and Updated: Updated the background, guidelines and recommendations, and evidence-based scientific references. Literature review did not necessitate any modifications to coverage criteria. The following edits were made for clarity: All CC edited for clarity and consistency

03/09/2022	Annual review: Updated background, guidelines and recommendations, and evidence-based scientific references. Addition to CC1a of "in patients with hypercalcemia", now reads "a) To assess for possible hyperparathyroidism in patients with hypercalcemia; OR"
03/03/2021	Annual review: Updated background, guidelines and recommendations, and evidence-based scientific references. Literature review did not necessitate any additional modifications to the CCs.
03/10/2020	Annual review: Updated background, guidelines and recommendations, and evidence-based scientific references. The E&I CC was changed to DNMCC with a preceding statement regarding a lack of available, published scientific literature. The literature review did not necessitate any additional modifications to the CCs.
03/01/2019	Initial Presentation

Pathogen Panel Testing

Policy Number: AHS – G2149 – Pathogen Panel Testing	Prior Policy Name and Number, as applicable:
Initial Presentation Date: 03/20/2017 Effective Date: 4/1/2025	

Policy Description

Infectious diseases can be caused by a wide range of pathogens. Conventional diagnostic methods like culture, microscopy with or without stains and immunofluorescence, and immunoassay often lack sensitivity and specificity and have long turnaround times. Panels for pathogens using multiplex amplified probe techniques and multiplex reverse transcription can detect and identify multiple pathogens in one test using a single sample (Palavecino, 2019).

Related Policies

Policy Number	Policy Title
AHS-M2057	Diagnosis of Vaginitis
AHS-M2097	Identification of Microorganisms using Nucleic Acid Probes
AHS-M2172	Onychomycosis Testing

Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

This policy is specific to testing in the outpatient setting. Criteria below do not apply to testing allowances in situations other than the outpatient setting.

1) For individuals with persistent diarrhea or diarrhea with signs or risk factors for severe disease (i.e., fever, bloody diarrhea, dysentery, dehydration, severe abdominal pain), multiplex PCR-based panel testing (up to **11** gastrointestinal pathogens [GIPs]) no more often than once every 7 days **MEETS COVERAGE CRITERIA**.

2) For individuals who are displaying signs and symptoms of a respiratory tract infection (i.e., temperature ≥ 102°F, pronounced dyspnea, tachypnea, tachycardia), multiplex PCR-based panel testing (up to **5** respiratory pathogens) **MEETS COVERAGE CRITERIA**.

3) Multiplex PCR-based panel testing of **12 or more** GIPs **DOES NOT MEET COVERAGE CRITERIA**.

4) Multiplex PCR-based panel testing of **6 or more** respiratory pathogens **DOES NOT MEET COVERAGE CRITERIA**.

5) Multiplex PCR-based panel testing of pathogens in cerebrospinal fluid (CSF) **DOES NOT MEET COVERAGE CRITERIA**.

6) Molecular detection-based panel testing of pathogens in the blood **DOES NOT MEET COVERAGE CRITERIA**.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

7) Molecular detection-based panel testing of urine pathogens for the diagnosis of urinary tract infections (e.g., GENETWORx Molecular PCR UTI Test) **DOES NOT MEET COVERAGE CRITERIA**.

8) Molecular-based panel testing to screen for or diagnose wound infections (e.g., GENETWORx PCR Wound Testing) **DOES NOT MEET COVERAGE CRITERIA**.

9) Molecular-based panel testing for general screening of microorganisms (e.g., MicroGenDX qPCR+ NGS) **DOES NOT MEET COVERAGE CRITERIA**.

Table of Terminology

Term	Definition
ACG	American College of Gastroenterology
ASCP	American Society for Clinical Pathology
BBB	Blood-brain barrier
BCID	Blood culture identification panel
BCSFB	Blood-cerebrospinal fluid barrier
CDC	Centers for Disease Control and Prevention
CDI	Clostridium difficile infections
CHEST	American College of Chest Physicians
CMS	Centers for Medicare and Medicaid Services

CNS	Central nervous system
CSF	Cerebrospinal fluid
DNA	Deoxyribonucleic acid
DOT	Days of therapy
EAEC	Enteroaggregative *Escherichia coli*
E. coli	*Escherichia coli*
EAU	European Association of Urology
EIEC	Enteroinvasive *Escherichia coli*
ESICM	European Society of Intensive Care Medicine
ETEC	Enterotoxigenic *Escherichia coli*
EUA	Emergency use authorization
FDA	Food and Drug Administration
GDH	Glutamate dehydrogenase
GI	Gastrointestinal
GIPs	Gastrointestinal pathogens
GPP	Gastrointestinal pathogen panel
HIV	Human immunodeficiency virus
HPV	Human papillomavirus infection
IDSA	Infectious Diseases Society of America
LAMP	Loop-mediated isothermal amplification
LCD	Local coverage determination
LDT	Laboratory developed test
ME	Meningitis/encephalitis
MRSA	*Methicillin resistant staphylococcus aureus*
MSSA	*Methicillin sensitive staphylococcus aureus*
NAAT	Nucleic acid amplification test
NICE	National Institute for Health and Care Excellence
NP	Nasopharyngeal
NPS	Nasopharyngeal swabs
PCR	Polymerase chain reaction
PLA	Proprietary laboratory analyses
PPA	Percent positive agreement
RNA	Ribonucleic acid
RP	Respiratory pathogen
RP2	Respiratory pathogen panel 2
RPP	Respiratory pathogen panel
RSV	Human respiratory syncytial virus
RT-PCR	Reverse transcriptase polymerase chain reaction
RV+	Respiratory virus plus nucleic acid test
RVP	Respiratory viral panel
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2

SCCM	Society of Critical Care Medicine
SHEA	Society for Healthcare Epidemiology of America
SOT	Solid organ transplant
SSTI	Skin and soft tissue infection
STEC	Shiga-toxin producing *Escherichia coli*
STX1	Shiga toxin 1
STX2	Shiga toxin 2
TEM-PCRTM	Target enriched multiplex polymerase chain reaction
UOS	Unit of service
UPEC	Uropathogenic *Escherichia coli*
UTI	Urinary tract infection
WGO	World Gastroenterology Organization
WHO	World Health Organization
WHO-RT-PCR	World Health Organization recommended reverse transcriptase polymerase chain reaction

Scientific Background

There has been a move in recent years towards employing molecular tests that use multiplex polymerase chain reaction (PCR) to simultaneously detect multiple pathogens associated with an infectious disease rather than one organism. These tests are usually offered as a panel for a particular infectious condition, such as sepsis and blood stream infections, central nervous system infections (for example, meningitis and encephalitis), respiratory tract infections, urinary tract infections or gastrointestinal infections. These assays are often more sensitive than conventional culture-based or antigen detection. The high diagnostic yield is particularly important when clinical samples are difficult to collect or are limited in volume (e.g., CSF). Multiplex PCR assays are also particularly beneficial when different pathogens can cause the same clinical presentation, thus making it difficult to narrow down the causative pathogen. Access to comprehensive and rapid diagnostic results may lead to more effective early treatment and infection-control measures. Disadvantages of multiplex PCR assays include high cost of testing and potential false negative results due to preferential amplification of one target over another (Palavecino, 2019).

The Centers for Medicare and Medicaid Services (CMS) report that the top target pathogens causing infections include *Salmonella*, *Campylobacter*, *Shigella*, *Cryptosporidium*, Shiga toxin producing *E. coli* non-O157 and Shiga toxin producing *E. coli* O157; these pathogens "represent the top 90-95% of foodborne infections [incidence of infection per 100,000 population]" (CMS, 2022).

Proprietary Testing

Gastrointestinal Pathogen Panel

Approximately 1.7 billion cases of childhood diarrheal disease occur worldwide every year, resulting in about 443,832 deaths in children younger than five years of age annually (WHO, 2024). The Centers for Disease Control and Prevention (CDC) has estimated that nearly 48 million cases of acute diarrheal infection occur annually in the United States, at an estimated cost upwards of $150 million (Scallan et al., 2011). Approximately 31 major pathogens acquired in the United States caused an estimated 9.4 million episodes of diarrheal illness, 55,961 hospitalizations, and 1,351 deaths each year. Additionally,

unspecified agents caused approximately 38 million episodes of foodborne illnesses and resulted in 71,878 hospitalizations and 1,686 deaths. Diarrhea can be classified as acute (lasting less than 14 days), persistent (14 and 30 days), and chronic (lasting for greater than a month) (Riddle et al., 2016). Further, healthcare and antibiotic associated diarrhea are mainly caused by toxin-producing *Clostridium difficile* causing more than 300,000 cases annually (CMS, 2022).

Acute infectious gastroenteritis is generally associated with other clinical features like fever, nausea, vomiting, severe abdominal pain and cramps, flatulence, bloody stools, tenesmus, and fecal urgency. A wide spectrum of enteric pathogens can cause infectious gastroenteritis, including bacteria such as *Campylobacter, Clostridium difficile, Salmonella, Shigella, Vibrio,* and *Yersinia*; viruses, such as Norovirus, Rotavirus, Astrovirus, and Adenovirus; and parasites, such as *Giardia, Entamoeba histolytica,* and *Cryptosporidium* (Riddle et al., 2016).

Stool culture is the primary diagnostic tool for a suspected bacterial infection, but it is time-consuming and labor intensive. Stool samples are collected and analyzed for various bacteria present in the lower digestive tract via cell culture; these bacteria may be normal or pathogenic (Humphries & Linscott, 2015). By identifying the type of bacteria present in a stool sample, a physician will be able to determine if the bacteria are causing gastrointestinal problems in an individual. However, stool culture has a low positive yield. Similarly, methods like electron microscopic examination and immunoassay that are used to diagnose viruses are labor intensive and need significant expertise (Zhang et al., 2015). Multiplex PCR-based assays have shown superior sensitivity to conventional methods for detection of enteric pathogens and are increasingly used in the diagnosis of infectious gastroenteritis. These assays have significantly improved workflow and diagnostic output in the diagnosis of gastrointestinal infections (Zhang et al., 2015). Several FDA-approved multiplex PCR assays are now commercially available. Some assays can detect only bacterial pathogens in stool, whereas others can detect bacterial, viral, and parasitic pathogens. The Strong-LAMP assay is a technique which uses PCR to detect *Strongyloides stercoralis* in stool and urine samples (Fernandez-Soto et al., 2016), although it is not yet widely available (La Hoz & Morris, 2019).

Proprietary panels are available for the assessment of gastrointestinal pathogens. BioFire Diagnostics offers an FDA-approved 22-target testing panel for the gastroenteritis, termed the BioFire FilmArray Gastrointestinal Panel. The panel's bacteria targets include *Campylobacter, Clostridium difficile, Plesiomonas shigelloides*, *Salmonella, Yersinia enterocolitica, Vibrio (parahaemolyticus, vulnificus,* and *cholerae),* and *Vibrio cholerae*. The panel's diarrheagenic *E. coli* and *Shigella* targets include Enteroaggregative *E. coli*, Enteropathogenic *E. coli*, Enterotoxigenic *E. coli*, Shiga-like toxin-producing *E. coli* stx1/stx2, *E. coli* O157, and *Shigella/*Enteroinvasive *E. coli*. The panel's parasite targets include *Cryptosporidium, Cyclospora cayetanensis, Entamoeba histolytica,* and *Giardia lamblia*. The panel's virus targets include Adenovirus F40/41, Astrovirus, Norovirus GI/GII, Rotavirus A, and Sapovirus (I, II, IV, and V) (BioFire, 2023b). The manufacturer claims a sensitivity of 98.5% and specificity of 99.2% for this test and states that results are available within one hour of testing. However, BioFire notes that the test has not been evaluated for immunocompromised patients (BioFire, 2023b).

The FDA-approved xTAG Gastrointestinal Pathogen Panel, developed by Luminex, can simultaneously identify multiple bacterial, viral, and parasitic nucleic acids in both fresh and frozen human stool samples. This test can provide results in as little as five hours, and can "detect and identify >90% of the causative bacterial, viral, and parasitic agents of gastroenteritis in the same day" (Luminex, 2023b). The xTAG Gastrointestinal Pathogen Panel is able to identify *Campylobacter*, *Clostridium difficile*, Toxin A/B, *Escherichia coli* O157, Enterotoxigenic *E.coli* (ETEC) LT/ST, Shiga-like Toxin producing *E.coli* (Banerjee et al.) stx1/stx2, *Salmonella, Shigella, Vibrio cholerae, Yersinia enterocolitica,* Adenovirus 40/41, Norovirus

GI/GII, Rotavirus A, *Cryptosporidium*, *Entamoeba histolytica*, and *Giardia* (Luminex, 2023b).

The Biocode Gastrointestinal Pathogen Panel is an FDA approved test that uses a 96-well microplate to simultaneously detect 17 diarrhea causing pathogens (*Campylobacter*, *Clostridium difficile* toxins A and B, *E. coli* O157, Enterotoxigenic *E. coli* LT/ST (ETEC), Enteroaggregative *E. coli* (EAEC), *Salmonella*, Shiga-like toxin producing *E. coli* stx1/stx2, *Shigella*/Enteroinvasive *E. coli*, *Vibrio/Vibrio parahemolyticus*, *Yersinia enterocolitica*, Adenovirus 40/41, Norovirus GI/GII, Rotavirus A, *Cryptosporidium*, *Entamoeba histolytica*, and *Giardia lamblia*) in stool samples (BioCode, 2024a). This rapid multiplex screening assay is low cost and may be helpful with infection control.

Respiratory Pathogen Panel

Upper respiratory tract infections (involving the nose, sinuses, larynx, pharynx, and large airways) can be caused by a variety of viruses and bacteria. These infections may lead to several different patient ailments such as the common cold, acute bronchitis, influenza, and respiratory distress syndromes. Regarding the common cold, the most common virus is rhinovirus; the bacteria that most commonly causes a sore throat (pharyngitis) is *Streptococcus pyogenes* (Thomas & Bomar, 2023). Lower respiratory tract infections occur in the lungs and any airways below the larynx. Lower respiratory infections include pneumonia, bronchitis, tuberculosis and bronchiolitis (Hansen et al., 2020).

Traditional methods used for the diagnosis of viral respiratory tract infections are direct antigen testing (non-immunofluorescent and immunofluorescent methods) and conventional and rapid cell culture (Ginocchio, 2007). These tests have several limitations including a slow turnaround time, low sensitivity, and labor-intensive processes. Acute respiratory infections may also be diagnosed by a simple respiratory exam, where the physician focuses on the patient's breathing and checks for fluid and inflammation in the lungs. Symptoms of a respiratory tract infection may include a stuffed nose, cough, fever, sore throat, headache, and difficulty breathing. Chest X-rays may be used to check for pneumonia, and blood/mucus samples may be used to confirm the presence of certain bacteria and/or viruses via cell culture. The doctor may also check the ears, nose, and throat. Treatment typically incorporates over the counter medications, rest, fluids, and antibiotics (if a bacterial infection is identified).

Considerable progress has been made in the development of molecular methods to detect multiple respiratory pathogens simultaneously. Molecular detection, including multiplex PCR assays, is currently the gold standard for viral respiratory diagnosis (Bonnin et al., 2016). Multiplex PCR-based assays are now commercially available to detect several viral pathogens like adenovirus, influenza A and respiratory syncytial virus as well as bacterial pathogens like *Mycoplasma pneumoniae, Chlamydophila pneumoniae,* and *Legionella pneumophila*. These tests are rapid, sensitive, specific, and the preferred testing method to identify most respiratory pathogens (Caliendo, 2011; Pammi, 2024; Yan et al., 2011). These tests may be a more reliable diagnostic test as they can be performed in just hours, do not require as large a volume of blood, and are not affected by antepartum antibiotics (Pammi, 2024).

BioFire has updated their FDA approved respiratory panel tests, the FilmArray RP and RP2, to become the FilmArray RP2.1 panel test. The new test, RP2.1, has added SARS-CoV-2 as a target compared to the previous versions of the respiratory panels (BioFire, 2023d). The prior FilmArray RP2.1 is able to detect 18 viral (Adenovirus, Coronavirus HKU1, Coronavirus NL63, Coronavirus 229E, Coronavirus OC43, Severe Acute Respiratory Syndrome Coronavirus 2, Human Metapneumovirus, Human Rhinovirus/Enterovirus, Influenza A, Influenza A/H1, Influenza A/H3, Influenza A/H1-2009, Influenza B, Parainfluenza Virus 1, Parainfluenza Virus 2, Parainfluenza Virus 3, Parainfluenza Virus 4, Respiratory Syncytial Virus) and 4 bacterial (*Bordetella parapertussis, Bordetella pertussis*, *Chlamydia pneumoniae* and *Mycoplasma*

pneumoniae) targets. This FilmArray RP2.1 panel test can detect the 22 targets in 45 minutes with a 97.1% sensitivity and 99.3% specificity (BioFire, 2023d).

GenMark Diagnostics has developed FDA-approved rapid ePlex® Respiratory Pathogen Panel (Uyeki et al.) and Respiratory Pathogen Panel 2 (RP2) tests. They can identify the most common bacterial and viral pathogens causing upper respiratory infections. The RP test can detect pathogens including Adenovirus, Coronavirus (229E, HKU1, NL63, OC43), Human Metapneumovirus, Human Rhinovirus/Enterovirus, Influenza A, Influenza A H1, Influenza A H1-2009, Influenza A H3, Influenza B, Parainfluenza 1, Parainfluenza 2, Parainfluenza 3, Parainfluenza 4, Respiratory Syncytial Virus A, Respiratory Syncytial Virus B, *Chlamydia pneumoniae,* and *Mycoplasma pneumoniae*. The RP2 test will detect the same pathogens along with SARS-CoV-2 (GenMark, 2023). The ePlex® Respiratory Pathogen Panel test was more efficient than a laboratory developed PCR assay resulting "in a significant decrease in time to result, enabling a reduction in isolation days in half of the patients," and increasing the identification of the causative pathogen (van Rijn et al., 2018).

The BioCode Respiratory Pathogen Panel is the FDA approved low-cost test that can simultaneously detect respiratory pathogens in nasopharyngeal swabs. This test is designed in a 96-well microplate format. The following 17 pathogens can be identified with this panel: Adenovirus, Coronavirus (229E, OC43, HKU1, and NL63), Human Metapneumovirus A/B, Influenza A, including subtypes H1, H1 2009 Pandemic, and H3, Influenza B, Parainfluenza 1, Parainfluenza 2, Parainfluenza 3, Parainfluenza 4, Respiratory Syncytial Virus A/B, Rhinovirus/Enterovirus, *Bordetella pertussis*, *Chlamydia pneumoniae* and *Mycoplasma pneumoniae* (BioCode, 2024b).

The NxTAG Respiratory Pathogen Panel, developed by Luminex, is able to simultaneously detect 20 pathogens (Influenza A, Influenza A H1, Influenza A H3, Influenza B, Respiratory Syncytial Virus A, Respiratory Syncytial Virus B, Rhinovirus/Enterovirus, Parainfluenza virus 1, Parainfluenza virus 2, Parainfluenza virus 3, Parainfluenza virus 4, Human Metapneumovirus, Adenovirus, Coronavirus HKU1, Coronavirus NL63, Coronavirus 229E, Coronavirus OC43, Human Bocavirus, *Chlamydophila pneumoniae* and *Mycoplasma pneumoniae*) in a single test. The CE Marked panel also detects *Legionella pneumophila* (Luminex, 2023a).

QIAGEN Science has developed the QIAstat-Dx Respiratory SARS-CoV-2 Panel, which is authorized by the FDA under an Emergency Use Authorization (EUA). It can detect the SARS-CoV-2 virus along with 20 other respiratory pathogens, including Adenovirus, Coronavirus 229E, Coronavirus HKU1, Coronavirus NL63, Coronavirus OC43, Human Metapneumovirus A+B, Influenza A, Influenza A H1, Influenza A H3, Influenza A H1N1/pdm09, Influenza B, Parainfluenza virus 1, Parainfluenza virus 2, Parainfluenza virus 3, Parainfluenza virus 4, Rhinovirus/Enterovirus, Respiratory Syncytial Virus A+B, *Bordetella pertussis, Chlamydophila pneumoniae, and Mycoplasma pneumoniae*. It is able to provide qualitative results within an hour and is for *in vitro* diagnostic use (QIAGEN, 2024). When compared with the currently WHO-recommended RT-PCR (WHO-RT-PCR), the QIAstat-Dx Respiratory Panel had a 97% agreement with the WHO-RT-PCR and a sensitivity of 100% and specificity of 93% (Visseaux et al., 2020).

Central Nervous System Panel

The brain is well protected from microbial invasion via the blood-brain barrier (BBB) and blood-cerebrospinal fluid barrier (BCSFB). Nonetheless, bacteria, fungi, viruses, and amoebae can infect the brain and the consequences are often fatal. Points of entry include the BBB, BCSFB, and the olfactory and trigeminal nerves (Dando et al., 2014). Meningitis, which is when the brain and/or spinal cord become inflamed, is typically caused by viral infections due to enteroviruses; other neurotropic viruses

include herpes simplex viruses, human cytomegalovirus, varicella-zoster virus, and rabies virus (Dando et al., 2014). In the United States, bacterial meningitis is most commonly caused by *Streptococcus pneumoniae*, group B *Streptococcus, Neisseria meningitidis, Haemophilus influenzae, Listeria monocytogenes, and Escherichia coli* (CDC, 2024c). Fungal meningoencephalitis, which is described as inflammation of the brain and surrounding membranes, is often caused by *Cryptococcus, Histoplasma, Blastomyces, Coccidioides, and Candida* (CDC, 2024e). Meningococcal meningitis is typically caused by *Neisseria meningitidis* (CDC, 2024a). Other types of pathogens may enter the central nervous system. The increasing use of molecular tests for the detection of pathogens in cerebrospinal fluid (CSF) has redefined the diagnosis and management of central nervous system (CNS) infections such as meningitis and encephalitis. However, it is important that test results correlate to the probability of infection. According to Petti and Polage (2019), the number of false-positive test results increase when the multiplex PCR tests are ordered in the absence of an elevated leukocyte count or elevated protein level in the CSF. Hence, the predictive value of the test increases when the tests are ordered only for those patients with a moderate to high pretest probability of having CNS infections based on clinical presentation and CSF findings (Petti & Polage, 2024).

The evaluation of meningitis routinely includes molecular testing, particularly when the patient is suspected to have viral meningitis. Although use of Gram stain and culture is the gold standard for diagnosis of bacterial meningitis, multiplex PCR assays may be useful as an adjunct, especially in patients who have already received antibiotic treatment. Other lab findings (for example, CSF cell count, glucose, and protein analyses) should be used as a screening method prior to the performance of molecular testing. Molecular assays for meningitis caused by fungi, parasites, rickettsia, and spirochetes are in development at this time (Petti & Polage, 2024).

Similarly, molecular testing of CSF is recommended when viral encephalitis, especially encephalitis due to Herpesviridae, is suspected. For other viral encephalitis, the clinical sensitivity and predictive value of multiplex-PCR assays is unknown. Therefore, a negative result does not exclude infection, and a combined diagnostic approach, including other methods like serology, may be necessary to confirm the diagnosis. Multiplex PCR-based assays may be useful in certain cases of bacterial meningitis, especially when a slow-growing or uncultivable bacterium like *Coxiella burnetti* is involved. Molecular assays for encephalitis caused by fungi, parasites, rickettsia, and spirochetes need to be investigated further and are not routinely available at this time (Petti & Polage, 2024).

The FDA approved BioFire FilmArray meningitis/encephalitis panel can provide information on 14 different pathogens in one hour. This test uses 0.2 mL of cerebrospinal fluid, and is able to detect bacteria (*Escherichia coli* K1, *Haemophilus influenzae*, *Listeria monocytogenes*, *Neisseria meningitidis*, *Streptococcus agalactiae*, and *Streptococcus pneumoniae*), viruses (Cytomegalovirus, Enterovirus, Herpes simplex virus 1, Herpes simplex virus 2, Human herpesvirus 6, Human parechovirus, and Varicella zoster virus) and yeast (*Cryptococcus neoformans/gattii*) (BioFire, 2023c). BioFire states that this panel has an overall sensitivity of 94.2% and a specificity of 99.8% (BioFire, 2023c).

Sepsis Panel

Sepsis, also known as blood poisoning, is the body's systemic immunological response to an infection. Sepsis occurs when an infection (in the lungs, skin, urinary tract or another area of the body) triggers a chain reaction in an individual (CDC, 2024b). Sepsis can lead to end-stage organ failure and death. Septic shock occurs when sepsis results in extremely low blood pressure and abnormalities in cellular metabolism. The annual incidence of severe sepsis and septic shock in the United States is 300 per 100,000 people; sepsis is "the most expensive healthcare problem in the United States" (Gyawali et al.,

2019).

Sepsis-related mortality remains high, and inappropriate antimicrobial and anti-fungal treatment is a major factor contributing to increased mortality (Liesenfeld et al., 2014). Blood culture is the standard of care for detecting bloodstream infections, but the method has several limitations (Lamy et al., 2020). Fastidious, slow-growing, and uncultivable organisms are difficult to detect by blood culture, and the test sensitivity decreases greatly when antibiotics have been given prior to culture. Additionally, culture and susceptibility testing may require up to 72 hours to produce results. Multiplex PCR assays of positive blood culture bottles have a more rapid turnaround time and are not affected by the administration of antibiotics. Faster identification and resistance characterization of pathogens may lead to earlier administration of the appropriate antibiotic, resulting in better outcomes, and may lessen the emergence of antibiotic-resistant organisms (Banerjee et al., 2015).

The T2Bacteria Panel is the first "FDA-cleared test to identify sepsis-causing bacteria directly from whole blood without the wait for blood culture" (T2Biosystems, 2024). This panel is able to identify 50% of all bloodstream infections, 90% of all ESKAPE bacteria (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa*, and *Escherichia coli*) pathogens, and 70% of all blood culture species identified in the emergency room with a 95% sensitivity and 98% sensitivity (T2Biosystems, 2024).

The Magicplex™ Sepsis Real-time Test by Seegene can identify more than 90 sepsis-causing pathogens with only 1 mL of whole blood. This test identifies both bacteria and fungi, as well as three drug resistance markers in only six hours (Seegene, 2020, 2023).

GenMark has developed three ePlex® Blood Culture Identification (BCID) Panels. These include the ePlex BCID-Gram Positive Panel (identifies 20-gram positive bacteria and four resistance genes), the ePlex BCID-Gran Negative Panel (identifies 21-gram negative bacteria and six resistance genes), and the ePlex BCID-Fungal Panel (identifies 15-fungal organisms) (GenMark, 2020).

BioFire has developed the FDA-cleared FilmArray Blood Culture Identification Panel (BCID). The original panel could identify 24 targets, but the newly expanded BCID2 panel can identify 43 targets. Targets include gram-positive bacteria (*Enterococcus faecalis, Enterococcus faecium, Listeria monocytogenes, Staphylococcus, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus lugdunensis, Streptococcus, Streptococcus agalactiae, Streptococcus pneumoniae, Streptococcus pyogenes*), gram-negative bacteria (*Acinetobacter calcoaceticus-baumannii complex, Bacteroides fragilis, Enterobacterales, Enterobacter cloacae complex, Escherichia coli, Klebsiella aerogenes, Klebsiella oxytoca, Klebsiella pneumoniae group, Proteus, Salmonella, Serratia marcescens, Haemophilus influenzae, Neisseria meningitidis, Pseudomonas aeruginosa, Stenotrophomonas maltophilia*), yeast (*Candida albicans, Candida auris, Candida glabrata, Candida krusei, Candida parapsilosis, Candida tropicalis, Cryptococcus neoformans/gattii)*, and antimicrobial resistance genes (BioFire, 2023a).

Urinary Tract Infection Panel

Urinary tract infections (UTIs) occur in the urinary system and can be either symptomatic or asymptomatic. UTIs can include cystitis, an infection of the bladder or lower urinary tract, pyelonephritis, an infection of the upper urinary tract or kidney, urosepsis, urethritis, and conditions such as bacterial prostatitis and epididymitis (Bonkat et al., 2023; Hooton & Gupta, 2024). Typically, in an infected person, bacteriuria and pyuria (the presence of pus in the urine) are present and can be present in both symptomatic and asymptomatic UTIs. A urine culture can be performed to determine the presence of

bacteria and to characterize the bacterial infection (Meyrier, 2024).

Panels comprising common UTI pathogens are now commercially available. Firms such as MicroGenDX and NovaDX offer panels consisting of many different pathogens involved in UTIs (MicroGenDX, 2019a; NovaDX, 2023). The NovaDX is a qPCR based test which can detect 17 pathogens including bacteria (*Acinetobacter baumannii, Citrobacter freundii, Enterobacter aerogenes, Enterobacter cloacae, Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Klebsiella oxytoca, Klebsiella pneumoniae, Morganella morganii, Proteus mirabilis, Proteus vulgaris, Providencia stuartii, Pseudomonas aeruginosa, Staphylococcus saprophyticus*, and *Streptococcus agalactiae*) and yeast (*Candida albicans*) (NovaDX, 2023).

Cardwell et al. (2016) evaluated the microbiology of UTIs in hospitalized adults. Approximately 308 patients were included, with a total of 216 identified pathogens. The authors separated patients into three groups; "community acquired (Group 1); recent healthcare exposure (Group 2); or a history of identification of an extended-spectrum beta lactamase (ESBL)-producing organism (Group 3)." *Escherichia coli* was found to be the most common pathogen, but the frequency differed between groups. Other commonly identified pathogens included *Pseudomonas aeruginosa* (Cardwell et al., 2016).

Medina and Castillo-Pino (2019) estimated the prevalence of certain pathogens in UTI (complicated or uncomplicated). The authors found that up to 75% of uncomplicated UTIs and up to 65% of complicated UTIs are caused by uropathogenic *Escherichia coli* (UPEC). Other commonly seen pathogens included *Enterococcus spp,* Group B Streptococcus, *K. pneumonia,* and *S. saprophyticus* (Medina & Castillo-Pino, 2019).

Wound Panel

Wounds (acute or chronic) are almost always colonized by microbes, thereby leading to a significant rate of infection. Panel testing many pathogens have been proposed as a method to quickly identify and therefore treat a wound infection (Armstrong & Meyr, 2024). These panels may be culture-based or nucleic acid-based; nucleic acid panels are typically touted for their speed compared to culture panels.

Firms, such as GenetWorx, Viracor, and MicroGenDX, offer comprehensive panels addressing many different common pathogens, resistance genes, and more. Genera, such as *Streptococcus, Enterococcus,* and *Staphylococcus* are frequent targets of these panels. Different combinations of panels are available (GenetWorx, 2024; MicroGenDX, 2019b; Viracor, 2024).

The Wounds Pathogen Panel by GenetWorx can identify 30 targets including bacteria, fungi, and viruses. Targeted pathogens include *Enterococcus faecalis*, *Enterococcus faecium, Methicillin Resistant Staphylococcus aureus* (MRSA), *Methicillin Sensitive Staphylococcus aureus* (MSSA), *Staphylococcus epidermidis, Streptococcus pyogenes* (Group A Strep), *Streptococcus agalactiae* (Group B Strep), *Streptococcus dysgalactiae* (Group C Strep), *Acinetobacter baumannii, Bacteroides fragilis, Bartonella henselea, Bartonella quintana, Enterobacter cloacae, Escherichia coli, Klebsiella pneumoniae, Klebsiella oxytoca, Morganella morganii, Proteus mirabilis, Pseudomonas aeruginosa, Bartonella Quintana, Serratia marcescens, Candida albicans, Candida glabrata, Candida parapsilosis, Candida dubliniensis, Candida tropicalis, Candida krusei, Tricophyton metagrophytes, Trichophyton rubrum, Aspergillus fumigatus, Mycobacterium fortuitum*, Herpes Simplex Virus 1, Herpes Simplex Virus 2, and Herpes Simplex Virus 3 (GenetWorx, 2024).

The Viracor Skin and Soft Tissue Infection Panel can identify 19 bacterial targets using TEM-PCRTM (Target Enriched Multiplex Polymerase Chain Reaction). These bacterial targets include *Acinetobacter*

baumannii, Bacteroides spp., Citrobacter freundii, Clostridium novyi/septicum, Clostridium perfringens, Enterobacter aerogenes, Enterobacter cloacae, Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Kingella kingae, Klebsiella spp., Morganella morganii, Proteus mirabilis, Proteus vulgaris, Staphylococcus aureus, MRSA- Meth. resistant S. aureus, Panton-Valentine leukocidin gene, Staphylococcus lugdunensis, Streptococcus pyogenes (Group A) and Pseudomonas aeruginosa. This test has not been approved by the FDA and has a two to three day turnaround time (Viracor, 2024).

Ray et al. (2013) described the incidence and microbiology of skin and soft tissue infections (SSTIs). The authors focused on members of a Northern California health plan, identifying 376262 patients with 471550 SSTIs. Approximately 23% of these infections were cultured, 54% of these cultures were pathogen-positive, and *Staphylococcus aureus* was found in 81% of these specimens. The researchers calculated the rate of diagnosed SSTIs to be 496 per 10000 person-years (Ray et al., 2013).

A comprehensive list of the main commercial pathogen panel tests mentioned above can also be found in the table below. This table was last updated on 03/27/2023.

Commercial Pathogen Panel Tests		
Type of Panel	**Name**	**Pathogens Identified**
Gastrointestinal	BioFire FilmArray Gastrointestinal Panel	22 targets including bacteria, parasites, and viruses
Gastrointestinal	Luminex xTAG Gastrointestinal Pathogen Panel	15 targets including bacteria, parasites, and viruses
Gastrointestinal	Biocode Gastrointestinal Pathogen Panel	17 targets including bacteria, parasites, and viruses
Respiratory	BioFire FilmArray Respiratory 2.1 (RP2.1) Panel	22 targets including viruses and bacteria
Respiratory	GenMark Diagnostics Rapid ePlex® Respiratory Pathogen Panel	17 targets including viruses and bacteria
Respiratory	GenMark Diagnostics Rapid ePlex® Respiratory Pathogen 2 Panel	18 targets including viruses and bacteria
Respiratory	BioCode Respiratory Pathogen Panel	17 targets including viruses and bacteria
Respiratory	Luminex NxTAG Respiratory Pathogen Panel	20 targets including viruses and bacteria
Respiratory	QIAGEN Sciences QIAstat-Dx Respiratory Pathogen Panel	20 targets including viruses and bacteria
Central Nervous System	BioFire FilmArray Meningitis/ Encephalitis Panel	14 targets including bacteria, viruses and yeast
Sepsis	T2Bacteria Panel	5 ESKAPE pathogens and potentially more targets

Sepsis	Magicplex™ Sepsis Real-time Test	90+ including bacteria and fungi
Sepsis	GenMark ePlex® Blood Culture Identification Panel (Gram-positive, Gram-negative and fungal)	56 bacteria and fungi
Sepsis	BioFire Blood Culture	43 targets including bacteria and yeast
Urinary Tract Infection	NovaDX UTI Test	17 targets including bacteria and yeast
Wound	GENETWORx PCR Wound Testing	30 targets including bacteria, fungi, mycobacteria, and viruses
Wound	Viracor Skin and Soft Tissue Infection Panel	19 bacterial targets

Clinical Utility and Validity

Several studies demonstrated the overall high sensitivity and specificity of the gastroenterology pathogen panels (Buss et al., 2015; Claas et al., 2013; Onori et al., 2014). Several studies have also indicated that gastrointestinal pathogen panels are more sensitive than culture, microscopy, or antigen detection, thus illustrating the potential of panels as a diagnostic tool for gastrointestinal infections (Buss et al., 2015; Couturier et al., 2011; Humphrey et al., 2016; Liu et al., 2014; Operario & Houpt, 2011). Zhang and colleagues concluded that using multiplex PCR assays in the work-up of infectious gastroenteritis has the potential to improve the diagnosis (Zhang et al., 2015).

Numerous studies have examined the clinical utility of the BioFire FilmArray GI Panel. Stockmann et al. (2015) focused on comparing the accuracy in detecting etiologic agents, particularly *Clostridioides difficile*, in stool specimen of pediatric patients with diarrhea between the FilmArray GI Panel with various standard laboratory methods performed at the discretion of the physician. They found that "a potential aetiologic agent was identified in 46% of stool specimens by standard laboratory methods and in 65% of specimens tested using the FilmArray GI Panel ($P<0.001$)." This FilmArray GI Panel was also able to detect concurrent infections by diarrheal pathogens other than *C. difficile*, including norovirus in 12% of supposed *C. difficile*-only testing cases. The FilmArray GI Panel also detected a pathogen in 63% of cases without additional *C. difficile* testing performed, and even detected *C. difficile* in 8% of those cases. These results proved the FilmArray GI Panel to be critical in detecting other diarrheal pathogens, and co-infections with other infectious diarrheagenic agents (Stockmann et al., 2015).

Similar results for the FilmArray GI Panel were found in another study for acute diarrhea. In conducting a prospective study, Cybulski et al. (2018) found that FilmArray detected pathogens at a higher rate than culture and at a faster time (35.3% in 18 hours versus 6.0% in 47 hours). This rapidity and accuracy also allowed patients to receive targeted therapy and facilitated quicker discontinuation of empirical antimicrobial therapy, demonstrating an improved clinical sensitivity with the FilmArray GI Panel when compared to culture (Cybulski et al., 2018). Beal et al. (2018) investigated the impact of submitting patient stool specimen for testing by the FilmArray GI panel ("cases") and compared overall findings with control patients from the year prior. The researchers concluded that this panel contributed to reducing the number of days on antibiotics (1.73 days among cases versus 2.12 days among controls), reducing "average length of time from stool culture collection to discharge" (3.4 days among cases vs 3.9 days among controls), and reducing overall health care cost by $293.61. They also found results like the previous studies on the FilmArray GI panel, with increased comprehensiveness of detectable pathogens,

and eliminating unnecessary testing and antibiotic use (Beal et al., 2018).

Axelrad et al. (2019) performed a retrospective comparative analysis of patients who underwent testing with the FilmArray GI panel from 2015-2017 and those who solely underwent conventional stool testing from 2012-2015. The FilmArray GI panel detected more pathogens (29.2% positive cases vs 4.1%) and reduced the need for additional endoscopic procedures and abdominal radiology imaging within 30 days following stool testing, as well as reduced chances of antibiotic prescription within 14 days following stool testing. The amassed literature communicates the great clinical utility and extended benefits from a multiplex PCR panel like the FilmArray GI Panel.

Zhan et al. (2020) performed a comparison of the BioFire FilmArray gastrointestinal panel and the Luminex xTAG Gastrointestinal Pathogen Panel for detecting diarrheal pathogens in China in a total of 243 diarrhea specimens. These two panels were highly consistent in detecting norovirus, rotavirus, and *Campylobacter*, but had low consistency in detecting *Cryptosporidium, Salmonella,* Shiga-toxin producing *Escherichia coli* (Banerjee et al.) and enterotoxigenic *Escherichia coli* (ETEC). The BioFire FilmArray panel was found to be more sensitive, but the Luminex xTAG Gastrointestinal Pathogen Panel was more specific. There appeared to be additional concern for how the Luminex xTAG Gastrointestinal Pathogen Panel yielded more false negatives when detecting ETEC as well (Zhan et al., 2020).

Jo et al. (2021) evaluated the use of the BioFire FilmArray gastrointestinal panel for pediatric patients with diarrhea. The authors compared the FilmArray GI panel results to conventional PCR for *E. Coli* and Allplex GI-Bacteria Assay results. A total 184 stool samples were tested, and it was found that "The BioFire GI Panel demonstrated a sensitivity of 100% for 12 targets and a specificity of >95% for 16 targets." The authors conclude that the FilmArray GI panel is useful for rapid identification of enteropathogenesis in pediatric patients (Jo et al., 2021).

Truong et al. (2021) investigated pediatric healthcare management before and after BioFire FilmArray gastrointestinal panel results were received. The study included 172 children, 120 of which had positive results. Based on the FilmArray GI panel results, the healthcare management plan changed for 23% of patients, including changes to antibiotic treatments, hospitalizations, room isolations, prescription changes, and test cancelations. The authors conclude that the FilmArray GI panel results impacted healthcare management, especially related to antibiotic treatment (Truong et al., 2021). Yoo at al. (2021) also studied the healthcare management of children with acute diarrhea using the BioFire FilmArray gastrointestinal panel. A total of 182 patients were included in the study. "A significant reduction in antibiotic use was observed in the prospective cohort compared to historical cohort, 35.3% vs. 71.8%; $p < 0.001$), respectively." The authors conclude that, likely due to the high positive rate and rapid reporting, the FilmArray GI panel was clinically beneficial for children, especially in reducing antibiotic use and enabling early precaution and isolation (Yoo et al., 2021).

Nijhuis et al. (2017) compared the GenMark Diagnostics ePlex Respiratory Pathogen panel with laboratory-developed real-time PCR assays for detecting respiratory pathogens. The study included 343 clinical specimens. The RP panel found an agreement of 97.4% with the real-time PCR assay regarding 464 pathogens found. The RP panel detected 17 more pathogens than the real-time PCR, showing that this panel could improve the efficiency of diagnostic "sample-to-answer testing" and cost-effectiveness, despite potentially costing more (Nijhuis et al., 2017).

van Asten et al. (2021) evaluated the performance of the GenMark Diagnostics ePlex Respiratory Pathogen panel and the QIAGEN Sciences QIAstat-Dx Respiratory Pathogen panel. The authors specifically studied the detection of three bacterial targets: *Legionella pneumophila, Mycoplasma*

pneumoniae and *Bordetella pertussis*. The study included 56 specimens taken from the lower respiratory tract, five of which were negative and the other 51 had previously tested positive on real-time PCR assays for the targets. "The QIAstat-Dx Respiratory Panel V2 (Uyeki et al.) assay detected all of the *L. pneumophila* and *B. pertussis* positive samples but only 11/15 (73.3 %) of the *M. pneumoniae* targets. The ePlex Respiratory Pathogen Panel (RPP) assay detected 10/14 (71.4 %) of the L. pneumophila targets, 8/12 (66.7 %) of the B. pertussis positive samples and 13/15 (86.7 %) of the M. pneumoniae targets." The authors concluded that the clinical performance of both panels depend on the bacterial lode and sample type (van Asten et al., 2021).

Mormeneo Bayo et al. (2022) compared real-time PCR with microscopy in detecting intestinal protozoa in children. The study used the Seegene Allplex Gastrointestinal panel for the real-time PCR. Five hundred stool samples were analyzed from children, 15 years of age and under, and grouped into two classifications based on if the children had or had not had clinical parasitosis. Based on microscopy, 6.2% of samples were positive. Based on real-time PCR, 51.2% of samples were positive. The authors concluded that "real-time PCR increases the detection of intestinal protozoa, being underdiagnosed by microscopy, especially D. fragilis, in which PCR is considered the most appropriate method for its detection" (Mormeneo Bayo et al., 2022).

Trujillo-Gómez et al. (2022) the diagnostic test accuracy of the FilmArray Meningitis/Encephalitis panel. The authors perfmored a systematic review of 19 studies containing a total of 11,251 participants, and performed a random-effects bivariate meta-analysis of diagnostic test accuracy. Using CSF/blood samples, the sensitivity was estimated to be 89.5% and the specificity was estimated to be 97.4%. Using the "final diagnosis adjudication based on clinical/laboratory criteria" the sensitivity was estimated to be 92.1% and the specificity was estimated to be 99.2%. The authors note that the certainty of evidence was low. The authors conclude that the FilmArray Meningitis/Encephalitis panel "may have acceptable-to-high sensitivities and high specificities for identifying bacteria, especially for *S.pneumoniae*, and viruses, especially for HSV-2, and enteroviruses" but suboptimal sensitivities for *L.monocytogenes, H.influenzae, E.coli*, and HSV-1 (Trujillo-Gómez et al., 2022).

Yoo et al. (2019) compared the Seegene Allplex Gastrointestinal, Luminex xTAG Gastrointestinal Pathogen Panel, and BD MAX Enteric Assays to determine which was the most efficient in detecting gastrointestinal pathogens from clinical stool samples. A total of 858 stool samples were used in this study. "The overall positive percentage agreements of Seegene, Luminex, and BD MAX were 94% (258 of 275), 92% (254 of 275), and 78% (46 of 59), respectfully. For *Salmonella*, Luminex showed low negative percentage agreement because of frequent false positives (n = 31) showing low median fluorescent intensity. For viruses, positive/negative percentage agreements of Seegene and Luminex were 99%/96% and 93%/99%, respectively" (Yoo et al., 2019). Overall, the authors suggest that these assays are promising in the detection of gastrointestinal pathogens simultaneously. Mahony et al. (2009) concluded that multiplex PCR-based testing was the most cost-effective strategy for the diagnosis of respiratory virus infections in children and resulted in better patient outcomes (shorter hospital stays) at lower costs (Mahony et al., 2009). Ginocchio et al. (2009) compared the sensitivities, specificities, positive predictive values, and negative predictive values of four different Influenza A diagnostic tests, including rapid antigen, direct immunofluorescence, viral culture, and PCR panel. The authors inferred that the PCR panel test provided the best diagnostic option with the highest sensitivity for the detection of all influenza strains and identified a significant number of additional respiratory pathogens (Ginocchio et al., 2009). Subramony et al. (2016) reported the use of multiplex PCR-based assays for respiratory viruses in hospitalized patients resulted in decreased healthcare resource utilization, including decreased use of antibiotics and chest radiographs (Subramony et al., 2016). Babady et al. (2018) evaluated a new panel of 19 viruses and two bacteria (ePlex Respiratory Panel) with 2908 samples by comparing it to BioFire

FilmArray. Overall agreement was >95% for all targets, and positive agreement ranged from 85.1% to 95.1%. Negative agreement ranged from 99.5% to 99.8% (Babady et al., 2018).

The Infectious Diseases Society of America (IDSA) stated that CSF RT-PCR can be one of the methods used for the diagnosis of rabies virus and enteroviral encephalitis (Tunkel et al., 2008). Several studies have evaluated the clinical impact of RT-PCR for the detection of enterovirus in the CSF of patients with aseptic meningitis (Ramers et al., 2000; Robinson et al., 2002; Stellrecht et al., 2002). These studies showed a reduction in unnecessary diagnostic and therapeutic intervention (for example, antibiotic use, ancillary tests, etc.), length of hospital stay, and hospital costs. Tzanakaki et al. (2005) evaluated a multiplex PCR assay for detection of *Neisseria meningitidis*, *Streptococcus pneumoniae,* and *Haemophilus influenzae* type b, and concluded that the test had high sensitivity (between 88% and 93.9%), an overall specificity and positive predictive value of 100%, and a negative predictive value >99% (Tzanakaki et al., 2005). Leber et al. (2016) evaluated the performance of a commercially available multiplex PCR-based panel for meningitis and encephalitis, and concluded that the test is a sensitive and specific aid in diagnosis of CNS infections and leads to improved patient outcomes (Leber et al., 2016). Another study compared the FilmArray meningitis/encephalitis (ME) panel by BioFire Diagnostics, which uses 0.2 mL of CSF to test for 14 pathogens in one hour (BioFire, 2023c), to traditional culture and PCR assay methods. The FilmArray ME panel "demonstrated an overall percent positive agreement (PPA) of 97.5% (78/80) for bacterial pathogens, 90.1% (145/161) for viruses, and 52% (26/50) for *Cryptococcus neoformans/C. gattii*. Despite the low overall agreement (52%) between the ME panel and antigen testing for detection of C. neoformans/C. gattii, the percent positive agreement of the FilmArray assay for *C. neoformans/C. gattii* was 92.3%" (Liesenfeld et al., 2014; Liesman et al., 2018). The ME panel has also been proven to aid in "decreasing the utilization of antibiotic therapy among pediatric patients admitted for concerns related to meningitis or encephalitis" (McDonald et al., 2020). Their research demonstrated that introducing the ME panel helped to reduce the days of therapy (DoT) from five days to three days and the number of inpatient days. Using the ME panel also decreased the empiric use of intravenous third generation cephalosporins and ampicillin for treatment independent of a respiratory viral pathogen diagnosis. Identifying the specific etiology guided more appropriate antibiotic therapy (McDonald et al., 2020).

The use of multiplex PCR assays to identify pathogens following positive blood culture can be faster than standard techniques involving phenotypic identification and antimicrobial susceptibility testing that is required up to 72 hours after the blood culture became positive (Liesenfeld et al., 2014). A prospective randomized controlled trial evaluating outcomes associated with multiplex PCR detection of bacteria, fungi, and resistance genes directly from positive blood culture bottles concluded that the testing led to more judicious antibiotic use (Banerjee et al., 2015). A study by Ward and colleagues compared the accuracy and speed of organism and resistance gene identification of two commercially available multiplex-PCR sepsis panels to conventional culture-based methods for 173 positive blood cultures. The researchers discovered that both the assays accurately identified organisms and significantly reduced the time to definitive results (on average, between 27.95 and 29.17 hours earlier than conventional method) (Ward et al., 2015). Another study assessed the diagnostic accuracy of a commercially available multiplex PCR-based assay for detecting infections among patients suspected of sepsis. They concluded that the test had high specificity with a modest sensitivity and had higher rule-in value than the rule-out value. If the patient had a positive result, a clinician can confidently diagnose sepsis and begin appropriate antimicrobial therapy while avoiding unwanted additional testing (Chang et al., 2013).

There are a few limitations with this type of testing. First, the level—detection or non-detection—of a microorganism does not necessarily imply a diagnosis. The tests can only describe the levels of microorganisms found in the environment, but additional information is required to make a diagnosis. Second, the scope of the 16S rRNA sequencing used in testing may be limited. Differences in regions

more specific than rRNA (such as surface antigens or individual toxin genes) cannot be resolved with this test. For example, the test cannot distinguish between a pathogenic *C. difficile* strain and a nonpathogenic one. Moreover, the tests report some of their targets at a genus level only, which means that these targets cannot be differentiated at the species level (Almonacid et al., 2017; Watts et al., 2017). Finally, the PCR technique can introduce errors during the amplification leading to incorrect detection. PCR enzymes may accidentally create "artefacts" or otherwise incorrect sequences causing the detection or measurement of the microorganisms to be inaccurate (V. Wintzingerode et al., 1997).

Aichinger et al. (2008) studied the diagnostic gain of repeat testing for *C. difficile*. "351 individuals were tested only twice by PCR (12.4% of individuals tested by PCR). There were 92 individuals (3.2% of individuals tested by PCR) who had three or more PCR tests performed within seven days. In 85 (92.4%) cases, results of all tests were negative. There were no individuals who had positive results following an initial negative test. For six individuals (6.5%), the results switched from an initial positive to a subsequent negative result, while one patient (1.1%) demonstrated only positive results. They found that the use of repeat testing is unnecessary" (Aichinger, 2008).

UroSwab is a urine-based proprietary test from Medical Diagnostics LLC. UroSwab is a real-time PCR test intended to detect numerous pathogens potentially involved in sexually transmitted and urological infections. This test uses a patient's urine, and the turnaround time is estimated at 24-72 hours. The results include whether a pathogen's presence was normal or abnormal and includes comments on what the pathogen's presence means (Medical Diagnostics, 2024a, 2024b).

McCarty et al. (2023) tested the performance and clinical utility of the GenMark ePlex Blood Culture Identification Gram-Negative Panel. The authors used "matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry on bacterial isolates" as a reference to compare results. In total, 98.1% (106/108) of the bacteria identified by MALDI were on the GenMark panel, and "valid tests (107/108, 99.1%) yielded results on average 26.7 h earlier" (McCarty et al., 2023).

Guidelines and Recommendations

American College of Gastroenterology (ACG)

American College of Gastroenterology (ACG) stated that "diarrheal disease by definition has a broad range of potential pathogens particularly well suited for multiplex molecular testing. Several well-designed studies show that molecular testing now surpasses all other approaches for the routine diagnosis of diarrhea. Molecular diagnostic tests can provide a more comprehensive assessment of disease etiology by increasing the diagnostic yield compared with conventional diagnostic tests" (Riddle et al., 2016). Furthermore, the ACG recommended that "traditional methods of diagnosis (bacterial culture, microscopy with and without special stains and immunofluorescence, and antigen testing) fail to reveal the etiology of the majority of cases of acute diarrheal infection. If available, the use of Food and Drug Administration-approved culture independent methods of diagnosis can be recommended at least as an adjunct to traditional methods. (Strong recommendation, low level of evidence)" (Riddle et al., 2016).

The ACG also notes:

- "Diagnostic evaluation using stool culture and culture-independent methods if available should be used in situations where the individual patient is at high risk of spreading disease to others, and during known or suspected outbreaks."

- "Stool diagnostic studies may be used if available in cases of dysentery, moderate–severe disease, and symptoms lasting >7 days to clarify the etiology of the patient's illness and enable specific directed therapy" (Riddle et al., 2016).

In 2013, the ACG made the following recommendations on diagnostic tests used for Clostridium difficile infections (Surawicz et al., 2013):

- "Only stools from patients with diarrhea should be tested for Clostridium difficile. (Strong recommendation, high-quality evidence)"
- "Nucleic acid amplification tests (NAAT) for C. difficile toxin genes such as PCR are superior to toxins A + B EIA testing as a standard diagnostic test for CDI. (Strong recommendation, moderate-quality evidence)"
- "Glutamate dehydrogenase (GDH) screening tests for C difficile can be used in two- or three-step screening algorithms with subsequent toxin A and B EIA testing, but the sensitivity of such strategies is lower than NAATs. (Strong recommendation, moderate-quality evidence)"
- "Repeat testing should be discouraged. (Strong recommendation, moderate-quality evidence)"
- "Testing for cure should not be done. (Strong recommendation, moderate-quality evidence)" (Surawicz et al., 2013).

Infectious Diseases Society of America (IDSA)

In 2013, the IDSA stated that "molecular diagnostics that detect microbial DNA directly in blood have achieved a modest level of success, but several limitations still exist. Based on available data, well-designed multiplex PCRs appear to have value as sepsis diagnostics when used in conjunction with conventional culture and routine antibiotic susceptibility testing" (Caliendo et al., 2013).

The IDSA published guidelines for the diagnosis and management of infectious diarrhea which state:

Stool testing should be performed for *Salmonella*, *Shigella*, *Campylobacter*, *Yersinia*, *C. difficile*, and STEC in people with diarrhea accompanied by fever, bloody or mucoid stools, severe abdominal cramping or tenderness, or signs of sepsis. However, other bacterial, viral, and parasitic agents should be considered regardless of symptoms. Any specimen testing positive for bacterial pathogens by culture independent diagnostics (such as an antigen based molecular assay) should be cultured in a clinical or public health laboratory if isolation was requested or required. Finally, clinical consideration should occur with interpretation of results of multi-pathogen NAATs as these tests only detect DNA and not necessarily pathogens (Shane et al., 2017).

The IDSA advises that repeat testing of gastrointestinal pathogen panels (GIP) utilizing multiplex NAATs is not considered medically necessary within seven days during the same period of diarrhea. (McDonald et al., 2018).

The IDSA acknowledges the availability of an FDA-approved multiplex PCR targeting 14 organisms for diagnosing encephalitis and meningitis, but the society states it "should not be considered a replacement for culture." The IDSA also notes that for gram-negative or gram-positive bacteria, bacterial culture is noted as the main diagnostic procedure (albeit at low sensitivity and optional). Regarding UTI, the IDSA only recommends nucleic acid testing for adenovirus and BK polyoma virus (Miller et al., 2018).

Regarding "wounds" (termed skin and soft tissue infections in the IDSA guideline), the IDSA typically recommends culture for most pathogens. Only a few strains of bacteria and viruses (such as *Staphylococcus aureus*, coagulase-negative staphylococci, *Enterococcus spp,* MRSA, and streptococci)

were recommended for nucleic acid testing with the majority of bacterial and fungal pathogens recommended for culture instead (Miller et al., 2018).

The IDSA recommends RT-PCR or other molecular tests over other influenza tests in hospitalized patients. RT-PCR tests targeting a panel of respiratory pathogens are recommended in hospitalized, immunocompromised patients (Uyeki et al., 2018).

The IDSA acknowledges that multiplex viral NAAT (potentially combined with bacterial NAAT) makes some clinical sense for immunocompromised and critically ill patients with pneumonia, as well as for those with exacerbations of airway disease. "These are situations where treatment of non–influenza viruses such as respiratory syncytial virus (RSV) or adenovirus may be considered (eg, in a stem-cell-transplant patient) and rapid test results are most likely to influence subsequent modifications of empiric broad-spectrum antibiotics" (Hanson et al., 2020). However, while the analytic sensitivity of multiplex NAAT decreases the likelihood that an important pathogen will be missed, enhanced detection can also complicate interpretation of results and available studies on the significance of mixed infections have reported variable results. IDSA notes that "additional studies are needed to understand whether coinfections portend poorer prognosis. . . High analytic sensitivity also translates to high negative-predictive values (ie, generally >97%, depending on prevalence), but there may be important differences among individual panel targets or across manufacturers. It is incumbent on clinicians and laboratorians to understand the test characteristics of each individual panel target, especially if the results inform antibiotic de-escalation in high-acuity settings. Even the largest multiplex panels do not detect all potential pathogens, and the optimal multiplex panel design remains a matter of debate. As a result, current tests are not yet a replacement for bacterial and fungal culture with antimicrobial susceptibility testing. Culture also remains essential for epidemiologic studies, vaccine-related decisions, and local antibiograms" (Hanson et al., 2020)

Global Wound Biofilm Expert Panel Consensus Guidelines

A Global Wound Biofilm Expert Panel have strongly agreed that "there are currently no routine diagnostic tests available to confirm biofilm presence" and that "the most important measure for future diagnostic tests to consider is indication of where the biofilm is located within the wound" (Schultz et al., 2017).

Society of Critical Care Medicine and the European Society of Intensive Care Medicine (SCCM)

A collaboration of the Society of Critical Care Medicine and the European Society of Intensive Care Medicine issued international guidelines for management of sepsis and septic shock. It states "in the near future, molecular diagnostic methods may offer the potential to diagnose infections more quickly and more accurately than current techniques. However, varying technologies have been described, clinical experience remains limited, and additional validation is needed before recommending these methods as an adjunct to or replacement for standard blood culture techniques" (Rhodes et al., 2017).

A 2020 update regarding "Management of Septic Shock and Sepsis-Associated Organ Dysfunction in Children" was published by the Society of Critical Care Medicine (SCCM), European Society of Intensive Care Medicine (ESICM), and the International Sepsis Forum. In it, they acknowledge the presence of new molecular technologies, but remark that they are "currently relatively expensive, are not sufficient for all pathogens and antibiotic sensitivities, and are not universally available" (Weiss et al., 2020).

National Institute for Health and Care Excellence (NICE)

The NICE states there is "insufficient evidence to recommend the routine adoption in the NHS of the integrated multiplex polymerase chain reaction tests, xTAG Gastrointestinal Pathogen Panel, FilmArray GI Panel and Faecal Pathogens B assay, for identifying gastrointestinal pathogens in people with suspected gastroenteritis." NICE acknowledges that the tests show promise but need further data on their clinical utility (NICE, 2017).

American Society for Microbiology/Association for Molecular Pathology/Association of Public Health Laboratories/College of American Pathologists/Infectious Diseases Society of America/Pan American Society for Clinical Virology

These societies made a joint statement regarding respiratory viral panels and noted three populations in which multiplex panels would be beneficial. Those populations were "immunocompromised hosts, adult patients appearing acutely ill who are potential hospital admissions, and critically-ill adult patients, particularly ICU patients" (American Society for Microbiology, 2017).

American College of Chest Physicians (CHEST)

The CHEST has recommended that outpatient adults with an acute cough and suspected pneumonia should not undergo routine microbiological testing because there is no need for such testing. However, testing may be considered if the results would change the therapeutic approach. Microbiological tests may include culture, serologic, and PCR testing (Hill et al., 2019).

Centers for Disease Control and Prevention

Regarding molecular tests that are commonly used for a *C. difficile* diagnosis, the CDC states that that "FDA-approved PCR assays are same-day tests that are highly sensitive and specific for the presence of a toxin-producing C. diff organism. . . Molecular assays can be positive for C. diff in asymptomatic individuals and those who do not have an infection. Patients with other causes of diarrhea might be positive, which leads to over-diagnosis and treatment. . . When using multi-pathogen (multiplex) molecular methods, read the results with caution as the pre-test probability of C. diff infection might be less" (CDC, 2024d).

Infectious Diseases Society of America (IDSA) and Society for Healthcare Epidemiology of America

The IDSA and SHEA have stated that the best-performing method for detecting patients with a greater risk of a *C. difficile* infection from a stool sample is to "Use a stool toxin test as part of a multistep algorithm (ie, glutamate dehydrogenase [GDH] plus toxin; GDH plus toxin, arbitrated by nucleic acid amplification test [NAAT]; or NAAT plus toxin) rather than a NAAT alone for all specimens received in the clinical laboratory when there are no pre-agreed institutional criteria for patient stool submission (Figure 2) (weak recommendation, low quality of evidence)" (McDonald et al., 2018). These guidelines also state that repeat testing (within seven days) should not be performed. Panel testing is not specifically mentioned in these guidelines (McDonald et al., 2018).

The European Association of Urology

The EAU published urological infections guidelines. For uncomplicated UTIs (recurrent UTIs, cystitis, pyelonephritis), the EAU does not mention molecular testing at any point of the treatment algorithm; instead, they recommend bacterial culture or dipstick testing for diagnosis and recommending against extensive workup. The EAU notes that antimicrobial susceptibility testing should be performed in all

cases of pyelonephritis, but their guidelines do not suggest any methods over another. In complicated UTIs, the EAU recommends urine culture to identify cases of clinically significant bacteriuria (Bonkat et al., 2023).

American Society of Transplantation Infectious Diseases Community of Practice

These guidelines focus on identifying infections in transplant patients. Their recommendations are as follows:

"For the diagnosis of SOT [solid organ transplant] recipients with suspected gastrointestinal infections," gastrointestinal multiplex molecular assays are recommended to identify *Cryptosporidium, Cyclospora,* and *Giardia* (La Hoz & Morris, 2019)

American Society for Clinical Pathology (ASCP, through ChoosingWisely)

The ASCP states "Do not routinely order broad respiratory pathogen panels unless the result will affect patient management." They further state that patient management may include "provid [ing] immediate diagnosis and potentially expedite management decisions" and list "rapid molecular or point of care tests for RSV, Influenza A/B, or Group A pharyngitis" as examples (ASCP, 2019).

Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: http://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

There are numerous FDA-approved pathogen panels. Additionally, many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
87154	Culture, typing; identification of blood pathogen and resistance typing, when performed, by nucleic acid (DNA or RNA) probe, multiplexed amplified probe technique including multiplex reverse transcription, when performed, per culture or isolate, 6 or more targets
87483	Infectious agent detection by nucleic acid (DNA or RNA); central nervous system pathogen (eg, Neisseria meningitidis, Streptococcus pneumoniae, Listeria, Haemophilus influenzae, E. coli, Streptococcus agalactiae, enterovirus, human parechovirus, herpes simplex virus type 1 and 2, human herpesvirus 6, cytomegalovirus, varicella zoster virus, Cryptococcus), includes multiplex reverse transcription, when performed, and multiplex amplified probe technique, multiple types or subtypes, 12-25 targets

CPT	Code Description
87505	Infectious agent detection by nucleic acid (DNA or RNA); gastrointestinal pathogen (eg, Clostridium difficile, E. coli, Salmonella, Shigella, norovirus, Giardia), includes multiplex reverse transcription, when performed, and multiplex amplified probe technique, multiple types or subtypes, 3-5 targets
87506	Infectious agent detection by nucleic acid (DNA or RNA); gastrointestinal pathogen (eg, Clostridium difficile, E. coli, Salmonella, Shigella, norovirus, Giardia), includes multiplex reverse transcription, when performed, and multiplex amplified probe technique, multiple types or subtypes, 6-11 targets
87507	Infectious agent detection by nucleic acid (DNA or RNA); gastrointestinal pathogen (eg, Clostridium difficile, E. coli, Salmonella, Shigella, norovirus, Giardia), includes multiplex reverse transcription, when performed, and multiplex amplified probe technique, multiple types or subtypes, 12-25 targets
87631	Infectious agent detection by nucleic acid (DNA or RNA); respiratory virus (eg, adenovirus, influenza virus, coronavirus, metapneumovirus, parainfluenza virus, respiratory syncytial virus, rhinovirus), includes multiplex reverse transcription, when performed, and multiplex amplified probe technique, multiple types or subtypes, 3-5 targets
87632	Infectious agent detection by nucleic acid (DNA or RNA); respiratory virus (eg, adenovirus, influenza virus, coronavirus, metapneumovirus, parainfluenza virus, respiratory syncytial virus, rhinovirus), includes multiplex reverse transcription, when performed, and multiplex amplified probe technique, multiple types or subtypes, 6-11 targets
87633	Infectious agent detection by nucleic acid (DNA or RNA); respiratory virus (eg, adenovirus, influenza virus, coronavirus, metapneumovirus, parainfluenza virus, respiratory syncytial virus, rhinovirus), includes multiplex reverse transcription, when performed, and multiplex amplified probe technique, multiple types or subtypes, 12-25 targets
87636	Infectious agent detection by nucleic acid (DNA or RNA); severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Coronavirus disease [COVID-19]) and influenza virus types A and B, multiplex amplified probe technique
87637	Infectious agent detection by nucleic acid (DNA or RNA); severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Coronavirus disease [COVID-19]) and influenza virus types A and B, and respiratory syncytial virus, multiplex amplified probe technique
0068U	Candida species panel (C. albicans, C. glabrata, C. parapsilosis, C. kruseii, C tropicalis, and C. auris), amplified probe technique with qualitative report of the presence or absence of each species Proprietary test: MycoDART-PCR™ dual amplification real time PCR panel for 6 Candida species Lab/Manufacturer: RealTime Laboratories, Inc/MycoDART, Inc
0086U	Infectious disease (bacterial and fungal), organism identification, blood culture, using rRNA FISH, 6 or more organism targets, reported as positive or negative with phenotypic minimum inhibitory concentration (MIC)-based antimicrobial susceptibility Proprietary test: Accelerate PhenoTest™ BC kit Lab/Manufacturer: Accelerate Diagnostics, Inc.
0109U	Infectious disease (Aspergillus species), real-time PCR for detection of DNA from 4 species (A. fumigatus, A. terreus, A. niger, and A. flavus), blood, lavage fluid, or tissue, qualitative reporting of presence or absence of each species Proprietary test: MYCODART Dual Amplification Real Time PCR Panel for 4 Aspergillus species Lab/Manufacturer: RealTime Laboratories/MycoDART, Inc

CPT	Code Description
0112U	Infectious agent detection and identification, targeted sequence analysis (16S and 18S rRNA genes) with drug-resistance gene Proprietary test: MicroGenDX qPCR & NGS For Infection Lab/Manufacturer: MicroGenDX
0115U	Respiratory infectious agent detection by nucleic acid (DNA and RNA), 18 viral types and subtypes and 2 bacterial targets, amplified probe technique, including multiplex reverse transcription for RNA targets, each analyte reported as detected or not detected Proprietary test: ePlex Respiratory Pathogen (Uyeki et al.) Panel Lab/Manufacturer: GenMark Diagnostics, Inc
0140U	Infectious disease (fungi), fungal pathogen identification, DNA (15 fungal targets), blood culture, amplified probe technique, each target reported as detected or not detected Proprietary test: ePlex® BCID Fungal Pathogens Panel Lab/Manufacturer: GenMark Diagnostics, Inc
0141U	Infectious disease (bacteria and fungi), gram-positive organism identification and drug resistance element detection, DNA (20 gram-positive bacterial targets, 4 resistance genes, 1 pan gram-negative bacterial target, 1 pan Candida target), blood culture, amplified probe technique, each target reported as detected or not detected Proprietary test: ePlex® BCID Gram-Positive Panel Lab/Manufacturer: GenMark Diagnostics, Inc
0142U	Infectious disease (bacteria and fungi), gram-negative bacterial identification and drug resistance element detection, DNA (21 gram-negative bacterial targets, 6 resistance genes, 1 pan gram-positive bacterial target, 1 pan Candida target), amplified probe technique, each target reported as detected or not detected Proprietary test: ePlex® BCID Gram-Negative Panel Lab/Manufacturer: GenMark Diagnostics, Inc
0152U	Infectious disease (bacteria, fungi, parasites, and DNA viruses), DNA, PCR and next-generation sequencing, plasma, detection of >1,000 potential microbial organisms for significant positive pathogens Proprietary test: Karius® Test Lab/Manufacturer: Karius Inc
0202U	Infectious disease (bacterial or viral respiratory tract infection), pathogen-specific nucleic acid (DNA or RNA), 22 targets including severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), qualitative RT-PCR, nasopharyngeal swab, each pathogen reported as detected or not detected Proprietary test: BioFire® Respiratory Panel 2.1 (RP2.1) Lab/Manufacturer: BioFire® Diagnostics
0223U	Infectious disease (bacterial or viral respiratory tract infection), pathogen-specific nucleic acid (DNA or RNA), 22 targets including severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), qualitative RT-PCR, nasopharyngeal swab, each pathogen reported as detected or not detected Proprietary test: QIAstat-Dx Respiratory SARS CoV-2 Panel Lab/Manufacturer: QIAGEN GmbH

CPT	Code Description
0225U	Infectious disease (bacterial or viral respiratory tract infection) pathogen-specific DNA and RNA, 21 targets, including severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), amplified probe technique, including multiplex reverse transcription for RNA targets, each analyte reported as detected or not detected Proprietary test: ePlex® Respiratory Pathogen Panel 2 Lab/Manufacturer: GenMark Diagnostics
0240U	Infectious disease (viral respiratory tract infection), pathogen-specific RNA, 3 targets (severe acute respiratory syndrome coronavirus 2 [SARS-CoV-2], influenza A, influenza B), upper respiratory specimen, each pathogen reported as detected or not detected Proprietary test: Xpert® Xpress CoV-2/Flu/RSV plus (SARS-CoV-2 and Flue targets) Lab/Manufacturer: Cepheid®
0241U	Infectious disease (viral respiratory tract infection), pathogen-specific RNA, 4 targets (severe acute respiratory syndrome coronavirus 2 [SARS-CoV-2], influenza A, influenza B, respiratory syncytial virus [RSV]), upper respiratory specimen, each pathogen reported as detected or not detected Proprietary test: Xpert® Xpress CoV-2/Flu/RSV plus (all targets) Lab/Manufacturer: Cepheid®
0321U	Infectious agent detection by nucleic acid (DNA or RNA), genitourinary pathogens, identification of 20 bacterial and fungal organisms and identification of 16 associated antibiotic-resistance genes, multiplex amplified probe technique Proprietary test: Bridge Urinary Tract Infection Detection and Resistance Test Lab/Manufacturer: Bridge Diagnostics
0323U	Infectious agent detection by nucleic acid (DNA and RNA), central nervous system pathogen, metagenomic next-generation sequencing, cerebrospinal fluid (CSF), identification of pathogenic bacteria, viruses, parasites, or fungi Proprietary test: Johns Hopkins Metagenomic Next-Generation Sequencing Assay for Infectious Disease Diagnostics Lab/Manufacturer: Johns Hopkins Medical Microbiology Laboratory
0369U	Infectious agent detection by nucleic acid (DNA and RNA), gastrointestinal pathogens, 31 bacterial, viral, and parasitic organisms and identification of 21 associated antibiotic-resistance genes, multiplex amplified probe technique Proprietary test: GI assay (Gastrointestinal Pathogen with ABR) Lab/Manufacturer: Lab Genomics LLC, Thermo Fisher Scientific
0370U	Infectious agent detection by nucleic acid (DNA and RNA), surgical wound pathogens, 34 microorganisms and identification of 21 associated antibiotic-resistance genes, multiplex amplified probe technique, wound swab Proprietary test: Lesion Infection (Wound) Lab/Manufacturer: Lab Genomics LLC, Thermo Fisher Scientific
0371U	Infectious agent detection by nucleic acid (DNA or RNA), genitourinary pathogen, semiquantitative identification, DNA from 16 bacterial organisms and 1 fungal organism, multiplex amplified probe technique via quantitative polymerase chain reaction (qPCR), urine Proprietary test: Clear UTI Lab/Manufacturer: Lifescan Labs of Illinois, Thermo Fisher Scientific

CPT	Code Description
0373U	Infectious agent detection by nucleic acid (DNA and RNA), respiratory tract infection, 17 bacteria, 8 fungus, 13 virus, and 16 antibiotic-resistance genes, multiplex amplified probe technique, upper or lower respiratory specimen Proprietary test: Respiratory Pathogen with ABR (RPX) Lab/Manufacturer: Lab Genomics LLC, Thermo Fisher Scientific
0374U	Infectious agent detection by nucleic acid (DNA or RNA), genitourinary pathogens, identification of 21 bacterial and fungal organisms and identification of 21 associated antibiotic-resistance genes, multiplex amplified probe technique, urine Proprietary test: Urogenital Pathogen with Rx Panel (UPX) Lab/Manufacturer: Lab Genomics LLC, Thermo Fisher Scientific
0441U	Infectious disease (bacterial, fungal, or viral infection), semiquantitative biomechanical assessment (via deformability cytometry), whole blood, with algorithmic analysis and result reported as an index Proprietary test: IntelliSep® test Lab/Manufacturer: Cytovale®
0442U	Infectious disease (respiratory infection), myxovirus resistance protein a (mxa) and c-reactive protein (crp), fingerstick whole blood specimen, each biomarker reported as present or absent Proprietary test: FebriDx® Bacterial/NonBacterial Point-ofCare Assay Lab/Manufacturer: Lumos Diagnostics, LLC, Lumos Diagnostics, LLC
0480U	Infectious disease (bacteria, viruses, fungi, and parasites), cerebrospinal fluid (CSF), metagenomic next-generation sequencing (DNA and RNA), bioinformatic analysis, with positive pathogen identification Proprietary test: Bacteria, Viruses, Fungus, and Parasite Metagenomic Sequencing, Spinal Fluid (MSCSF) Lab/Manufacturer: Mayo Clinic, Laboratory Developed Test
0504U	Infectious disease (urinary tract infection), identification of 17 pathologic organisms, urine, realtime PCR, reported as positive or negative for each organism Proprietary test: Urinary Tract Infection Testing Lab/Manufacturer: NxGen MDx LLC
0528U	Lower respiratory tract infectious agent detection, 18 bacteria, 8 viruses, and 7 antimicrobial resistance genes, amplified probe technique, including reverse transcription for RNA targets, each analyte reported as detected or not detected with semiquantitative results for 15 bacteria Proprietary Test: BIOFIRE® FILMARRAY® Pneumonia (PN) Panel Lab/Manufacturer: bioMérieux, bioMérieux

Procedure codes appearing in policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

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Revision History

Revision Date	Summary of Changes
12/04/2024	Off-cycle coding modification: Added CPT code 0202U, 0223U, 0225U; added new CPT code 0528U (effective date 01/01/2025)

Pediatric Preventive Screening

Policy Number: AHS – G2042 – Pediatric Preventive Screening	Prior Policy Name and Number, as applicable: G2042 – Preventive Screening in Children and Adolescents
Initial Presentation Date: 11/16/2015 Revision date: 03/06/2024	

Policy Description

Preventive screening is a healthcare service with the goal of illness prevention and health management. According to the American College of Preventive Medicine (ACPM, 2023), "preventive medicine focuses on the health of individuals, communities, and defined populations. Its goal is to protect, promote, and maintain health and well-being and to prevent disease, disability, and death."

Pediatric preventive screening guidelines provide evidence-driven guidance for preventive care screenings and well-child visits. Bright Futures is a "national health promotion and prevention initiative, led by the American Academy of Pediatrics and supported by the Maternal and Child Health Bureau, Health Resources and Services Administration (AAP, 2021a).

This policy refers to laboratory-based preventive screening tests performed on individuals newborn through age 18 years, except for newborn screening for genetic disorders. The World Health Organization (WHO) defines an adolescent as any person between the age of 10 and 19 (WHO, 2023).

For guidance on screening for diabetes in the pediatric population, please refer to policy AHS-G2006-Diabetes Mellitus Testing. For guidance on testing for thyroid disease in the pediatric population, please refer to AHS-G2045-Thyroid Disease Testing. For guidance on screening for sexually transmitted infections in the pediatric population, please refer to AHS-G2157-Diagnostic Testing of Common

Sexually Transmitted Infections. For guidance on screening for human immunodeficiency virus in the pediatric population, please refer to policy AHS-M2116-Human Immunodeficiency Virus (HIV).

Terms such as male and female are used when necessary to refer to sex assigned at birth.

Related Policies

Policy Number	Policy Title
AHS-G2006	Diabetes Mellitus Testing
AHS-G2045	Thyroid Disease Testing
AHS-G2050	Cardiovascular Disease Risk Assessment
AHS-G2157	Diagnostic Testing of Common Sexually Transmitted Infections
AHS-M2116	Human Immunodeficiency Virus (HIV)

Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) When it follows all applicable federal and state law recommendations, a newborn screening panel **MEETS COVERAGE CRITERIA**.

2) For all newborns, screening for hyperbilirubinemia **MEETS COVERAGE CRITERIA**.

3) For all newborns, screening for congenital hypothyroidism utilizing serum thyroxine (T4) and/or thyroid-stimulating hormone (TSH) **MEETS COVERAGE CRITERIA**.

4) For all newborns, screening for sickle cell disease **MEETS COVERAGE CRITERIA**.

5) Blood lead screening **MEETS COVERAGE CRITERIA** for **any** of the following situations:
   a) For individuals ages 12 months to 2 years.
   b) For individuals ages 6 months to 6 years who have an increased risk for lead exposure (see Note 1).

6) Screening for anemia with hemoglobin or hematocrit determination **MEETS COVERAGE CRITERIA** for **any** of the following situations:
   a) For all individuals who are 12 months of age.
   b) For individuals 4 months and older who are at risk for iron deficiency (see Note 2).

7) For individuals 1 month of age or older who are at increased risk of contracting tuberculosis (see Note 3), tuberculosis screening **MEETS COVERAGE CRITERIA**.

8) Screening for dyslipidemia using a fasting lipid profile **or** a non-fasting non-HDL-C **MEETS COVERAGE CRITERIA** in **any** of the following situations:
   a) Annually for children and adolescents who are at increased risk due to personal history or family history (see Note 4).
   b) Once for all children and adolescents during each of the following age periods:

i) For individuals 9 – 11 years of age.

ii) For individuals 17 years of age.

NOTES:

Note 1: Lead exposure risk factors for children as defined by the CDC: living or spending time in a house or building built before 1978; growing up in a low-income household; being a recent immigrant, refugee or recently adopted from less developed countries; living or spending time with a person who works with lead or has hobbies that expose them to lead (CDC, 2023c).

Note 2: Iron deficiency risk factors for children as defined by the AAP: history of prematurity or low birth weight; exposure to lead; exclusive breastfeeding beyond 4 months of age without supplemental iron; weaning to whole milk or complementary foods that do not include iron-fortified cereals or foods naturally rich in iron, feeding problems, poor growth, and inadequate nutrition (Baker et al., 2010).

Note 3: TB risk factors for children as defined by the AAP: close contact with a person with or suspected to have infectious tuberculosis; radiographic or clinical findings suggestive of TB; HIV infection or considered at risk for HIV infection; being of foreign birth (especially if born in Asia, Africa or Latin America, countries of the former Soviet Union) or is a refugee, or immigrant; contact with HIV infected, homeless, nursing home residents, institutionalized or incarcerated individuals, illicit drug users or migrant farm workers; having a depressed immune system; living or has lived in a "high risk for tuberculosis" area; participating in significant travel to countries with endemic infections (AAP, 2022; Nolt et al., 2021).

Note 4: Dyslipidemia risk factors for children as defined by the AAP: pediatric patient family history includes family members with CVD or dyslipidemia that are ≤55 years of age for men and ≤65 years of age for women; pediatric patients who have an unknown family history or other CVD risk factors such as being overweight (BMI ≥ 85th percentile, <95th percentile), obesity (BMI ≥ 95th percentile), hypertension (blood pressure ≥ 95th percentile), cigarette smoking, or diabetes mellitus (Daniels et al., 2008).

Table of Terminology

Term	Definition
AACE	American Association of Clinical Endocrinologists
ACE	American College of Endocrinology
AAFP	American Academy of Family Physicians
AAP	American Academy of Pediatrics
ACA	Affordable Care Act
ACPM	American College of Preventive Medicine
ADA	American Diabetes Association
CDC	Centers for Disease Control and Prevention
CLIA '88	Clinical Laboratory Improvement Amendments of 1988
CMS	Centers for Medicare and Medicaid
FDA	Food and Drug Administration
G6PD	Glucose 6-phosphate dehydrogenase deficiency
HBV	Hepatitis B virus
HHS	Health and Human Services

HIV	Human immunodeficiency virus
HRSA	Health Resources and Services Administration
LDTs	Laboratory-developed tests
LP(a)	Lipoprotein a
NLA	National Lipid Association
NSMBB	Newborn screening and molecular biology branch
NSQAP	Newborn screening quality assurance program
RUSP	Recommended Uniform Screening Panel
TSH	Thyroid stimulating hormone
USPSTF	United States Preventive Services Task Force
WHO	World Health Organization

Scientific Background

The annual "wellness visit" or checkup visit to a primary care provider has been a common component of routine health care for several decades. Providers typically review an individual's personal history and family history, perform a physical examination, and run a battery of tests during the annual checkup. The types and number of tests performed can vary widely among providers.

Screening (checking for disease when there are no symptoms) can improve the likelihood of early detection and therefore also prognosis (NCI, 2023). The characteristics of a disease or condition, such as significant effects of an untreated disease, high prevalence in healthy populations, and utility of preclinical detection, can make a condition a good candidate for screening. Newborns and adolescents are more susceptible to certain conditions than adults and, consequently, are recommended for different screenings. For example, infants are typically screened for hyperbilirubinemia, although this condition is not seen as frequently in older children or adults. Schools will often be responsible for the screening of certain conditions, including scoliosis (Kelly, 2023).

Newborn screening is provided to healthy populations to identify newborns that require further testing. Each state handles newborn screening according to predetermined mandates. The United States Secretary of Health and Human Services has established the Recommended Uniform Screening Panel (RUSP) which provides a list of conditions that should be screened, including cystic fibrosis and phenylketonuria. A blood sample is typically taken from the heel of the newborn around the time of hospital discharge (Kemper, 2021). Most of these conditions are identified with tandem mass spectrometry or high pressure liquid chromatography, which are both well-validated (HRSA, 2018).

Screening in children and adolescents is also critical. Some of these screenings may not have apparent benefits for many years or even until adulthood, and the American Academy of Pediatrics (AAP) emphasizes that these preventive screenings have an additive effect (AAP, 2017). Conditions, such as lead poisoning or significant dyslipidemia, may cause irreversible damage during child development, and as such it is crucial to screen for these conditions. Due to the enormous variation in children and families, the AAP provides many recommendations in the form of a periodicity schedule; this schedule is meant for children "who are receiving competent parenting, have no manifestations of any important health problems, and are growing and developing in a satisfactory fashion." The AAP notes that developmental, psychosocial, or chronic issues may require additional counseling or treatment visits alongside the preventive care visits (AAP, 2017).

The Bright Futures initiative was started in 1990 by the Maternal and Child Health Bureau to improve the health of children and prevent disease. The AAP partnered with Bright Futures, and these organizations have now issued joint guidelines and recommendations related to the screening of children and adolescents for common preventable and treatable disorders. The recommendations are age-related and aligned with the standard timing of medical visits for children (AAP, 2021a).

Clinical Utility and Validity

The AAP has noted a lack of strong evidence to support pediatric preventive screening for numerous conditions. However, the AAP has emphasized that "lack of evidence does not mean a lack of effectiveness" and has ensured that their recommendations have adequately assessed the benefit of screening against potential harm (AAP, 2017).

The Centers for Disease Control and Prevention (CDC) estimates the number of newborn screenings to be four million a year in the United States (CDC, 2019a). The CDC performed a study assessing the number of conditions diagnosed because of screening newborns and identified approximately 12,500 diagnoses found due to the newborn screening programs, equaling approximately one out of 4000 live births. Severe disorders are identified in approximately 5,000 newborns each year (CDC, 2019a). At the time of the study, the core screening panels consisted of 29 core conditions. The five most commonly diagnosed conditions were (in order): hearing loss, primary congenital hypothyroidism, cystic fibrosis, sickle cell disease, and medium-chain acyl-CoA dehydrogenase deficiency. The CDC estimated congenital hearing loss to occur in one to three live births out of 1000. Finally, the CDC estimated the cost of the newborn screening program to be about $30 per infant, or $120 million (CDC, 2012). The CDC has also developed a newborn screening and molecular biology branch (NSMBB) and a newborn screening quality assurance program (NSQAP) that assists in the development of analytical methods to measure substances in dried blood spots. Certified materials for newborn screening tests are also produced by this branch (CDC, 2019b).

Guidelines and Recommendations

The American Academy of Pediatrics (AAP) and Bright Futures Recommendations for Preventive Pediatric Health Care

The American Academy of Pediatrics (AAP) (through Bright Futures) recommendations include the following screenings. The Bright Futures/AAP Periodicity Schedule describes the screenings, assessments, physical examinations, procedures, and timing of anticipatory guidance recommended for each age-related visit. Below are the laboratory-related screening recommendations:

- Newborn blood and bilirubin
- Anemia risk assessment at four months, test at 12 months, and further risk assessments at each subsequent visit with appropriate action to follow, if positive.
- Lead screening at six, nine, 12, 18, and 24 months and then once annually from three to six years, if indicated
- Tuberculosis screening at one, six, 12, and 24 months, and then annually thereafter starting at three years old, if indicated
- Dyslipidemia screening at 24 months and then every two years starting at four years old; AAP also recommends screening at least once between ages nine and 11 and between 17 and 21. Annual risk assessments starting at age 12 up to age 16 are recommended, with appropriate action to follow, if positive.

- STI/HIV screening annually starting at 11 years old, with at least one HIV screening between 15 and 21 (AAP, 2021b, 2023).

Many of these recommendations were based on the USPSTF's recommendations (AAP, 2017).

The AAP has also released a policy statement on targeted testing for lead. The AAP recommends targeted testing for lead in immigrant, refugee, or internationally adopted children at time of arrival (AAP, 2016).

The Advisory Committee on Heritable Disorders in Newborns and Children recommendations are included in the Bright Futures' periodicity table. The committee recommends that every newborn screening program include a Recommended Uniform Screening Panel (RUSP) that screens for 35 core disorders and 26 secondary disorders (RUSP, 2023). Required screenings vary by state.

The core disorders are as follows: Propionic Acidemia, Methylmalonic Acidemia, (methylmalonyl-CoA mutase) Methylmalonic Acidemia, (Cobalamin disorders) Isovaleric Acidemia, 3-Methylcrotonyl-CoA Carboxylase Deficiency, 3-Hydroxy-3-Methyglutaric Aciduria, Holocarboxylase Synthase Deficiency, ß-Ketothiolase Deficiency, Glutaric Acidemia Type I, Carnitine Uptake Defect/Carnitine Transport Defect, Medium-chain Acyl-CoA Dehydrogenase Deficiency, Very Long-chain Acyl-CoA Dehydrogenase Deficiency, Long-chain L-3 Hydroxyacyl-CoA Dehydrogenase Deficiency, Trifunctional Protein Deficiency, Argininosuccinic Aciduria, Citrullinemia, Type I, Maple Syrup Urine Disease, Homocystinuria, Classic Phenylketonuria, Tyrosinemia Type I, Primary Congenital Hypothyroidism, Congenital adrenal hyperplasia, S,S Disease (Sickle Cell Anemia), S, βeta-Thalassemia, S,C Disease, Biotinidase Deficiency, Critical Congenital Heart Disease, Cystic Fibrosis, Classic Galactosemia Glycogen Storage Disease Type II (Pompe), Hearing Loss, Severe Combined Immunodeficiencies, Mucopolysaccharidosis Type 1, X-linked Adrenoleukodystrophy, Spinal Muscular Atrophy due to homozygous deletion of exon 7 in *SMN1*.

The secondary disorders are as follows: Methylmalonic acidemia with homocystinuria, Malonic acidemia, Isobutyrylglycinuria, 2-Methylbutyrylglycinuria, 3-Methylglutaconic aciduria, 2-Methyl-3-hydroxybutyric aciduria, Short-chain acyl-CoA dehydrogenase deficiency, Medium/short-chain L-3-hydroxyacylCoA dehydrogenase deficiency, Glutaric acidemia type II, Medium-chain ketoacyl-CoA thiolase deficiency, 2,4 Dienoyl-CoA reductase deficiency, Carnitine palmitoyltransferase type I deficiency, Carnitine palmitoyltransferase type II deficiency, Carnitine acylcarnitine translocase deficiency, Argininemia, Citrullinemia type II, Hypermethioninemia, Benign hyperphenylalaninemia, Biopterin defect in cofactor biosynthesis, Biopterin defect in cofactor regeneration, Tyrosinemia, type II, Tyrosinemia, type III, Various other hemoglobinopathies, Galactoepimerase deficiency, Galactokinase deficiency, T-cell related lymphocyte deficiencies (Children, 2020).

There is also another category set forth by the RUSP—conditions for which newborn screening is not indicated. These include conditions that do not have adequate testing or did not meet other criteria in the RUSP's review. These conditions are as follows: Krabbe disease, Pompe disease, Lysosomal storage diseases, Creatine transport defect, Fabry disease, X-linked adrenoleukodystrophy, Hurler-Scheie disease, Biliary atresia, Smith-Lemli-Opitz syndrome, Congenital disorder of glycosylation type Ib, Fragile X syndrome, Duchenne and Becker muscular dystrophy, Congenital Cytomegalovirus infection, α1-Antitrypsin deficiency, Carbamylphosphate synthetase deficiency, Adenosine deaminase deficiency, Turner syndrome, Arginine: glycine amidinotransferase deficiency, Neuroblastoma, Diabetes mellitus, insulin dependent, Wilson disease, Guanidinoacetate methyltransferase deficiency, Ornithine transcarbamylase deficiency, Carnitine palmitoyltransferase IB deficiency (muscle), Familial hypercholesterolemia (heterozygote), Congenital Toxoplasmosis, Severe combined immunodeficiency,

Neonatal hyperbilirubinemia (Kernicterus), Glucose 6-phosphate dehydrogenase deficiency (G6PD) (HHS, 2023).

"Secondary" disorders refer to a class of conditions that are "part of the differential diagnosis of a core panel condition." The core disorders refer to conditions appropriate for newborn screening; they all "have specific and sensitive screening tests, a sufficiently well understood natural history, and available and efficacious treatments." Although states ultimately decide which conditions to screen for in their newborn screening programs, this list from the Department of Health and Human Services provides some standardization to those programs (HHS, 2023).

American Association of Clinical Endocrinologists (AACE) and American College of Endocrinology (ACE)

The 2017 AACE and ACE guidelines for Management of Dyslipidemia and Prevention of Cardiovascular Disease Recommend the following for children:

- "In children at risk for FH (e.g., family history of premature cardiovascular disease or elevated cholesterol), screening should be at 3 years of age, between 9 and 11, and at age 18" (Jellinger et al., 2017).
- "Screen adolescents older than 16 years every 5 years or more frequently if they have ASCVD risk factors, have overweight or obesity, have other elements of the insulin resistance syndrome, or have a family history of premature ASCVD" (Jellinger et al., 2017).

American Diabetes Association (ADA)

The ADA standards of Medical Care in Diabetes document state the following recommendations for children and adolescents' dyslipidemia testing:

- "Initial lipid profile should be performed soon after diagnosis, preferably after glycemia has improved and age is ≥2 years. If initial LDL cholesterol is ≤100 mg/dL (2.6 mmol/L), subsequent testing should be performed at 9-11 years of age. Initial testing may be done with a nonfasting non-HDL cholesterol level with confirmatory testing with a fasting lipid panel" (ADA, 2023).
- "If LDL cholesterol values are within the accepted risk level (<100 mg/dL [2.6 mmol/L]), a lipid profile repeated every 3 years is reasonable" (ADA, 2023).

United States Preventive Services Task Force (USPSTF)

The USPSTF recommends screening for Hepatitis B virus (HBV) in adolescents and adults who are at an increased risk for infection (Grade B) (USPSTF, 2020c). The USPSTF recommends screening for hepatitis C virus (HCV) infection in adults aged 18 to 79 years (Grade B) (USPSTF, 2020a).

In children and adolescents 20 years or younger, the USPSTF concludes that "the current evidence is insufficient to assess the balance of benefits and harms of screening for lipid disorders" (USPSTF, 2016).

The USPSTF recommends screening for syphilis in adolescents who have ever been sexually active and are at increased risk for syphilis infection. The USPSTF continues to recommend screening for syphilis in nonpregnant persons who are at increased risk for infection (USPSTF, 2022).

The USPSTF recommends screening for chlamydia and gonorrhea in all sexually active women ages 24 and under (Grade B) (USPSTF, 2021).

The USPSTF has stated that there is insufficient evidence to assess the balance of benefits and harms of screening for elevated blood lead levels in asymptomatic children ages 1-5 years (Cantor et al., 2019).

The USPSTF recommends screening adolescents 15 and older for HIV infection. Adolescents under 15 but who are at increased risk should also be screened (Grade A) (Chou et al., 2019; USPSTF, 2019).

The USPSTF has deemed the current evidence insufficient for children ages 6-24 months to be screened for iron deficiency anemia (Siu, 2015).

The USPSTF recognized the importance of screening for hemoglobinopathies in newborns including sickle cell disease, but will not update this 2007 recommendation (USPSTF, 2007).

The USPSTF recognized the importance of screening for congenital hypothyroidism in newborns in 2008, but will not update this recommendation (USPSTF, 2008a).

The USPSTF recognized the importance of screening for phenylketonuria in newborns, but will not update this 2008 recommendation (USPSTF, 2008b).

The USPSTF concludes that the current evidence is insufficient to assess the balance of benefits and harms of screening for primary hypertension in asymptomatic children and adolescents to prevent subsequent cardiovascular disease (Moyer, 2013). A 2020 recommendation statement by the USPSTF confirmed that the current evidence is insufficient to assess the balance of benefits and harms of screening for high blood pressure in children and adolescents (in general) (USPSTF, 2020b).

Centers for Disease Control and Prevention

The CDC acknowledges the Bright Futures and USPSTF recommendations for pediatric preventive screening, including HIV screening (CDC, 2018, 2020). On May 14, 2021, the CDC updated its blood lead reference value (BLRV) from 5 µg/dL to 3.5 µg/dL in response to a recommendation from the Lead Exposure and Prevention Advisory Committee (LEPAC). The BLRV is a metric used to identify children with blood lead levels that are higher than most (97.5th percentile) other children's levels (CDC, 2022).

The CDC recommends Healthcare providers use the "Catch-up Immunization schedule" to immunize children who are more than one month behind in immunizations (CDC, 2023a).

(CDC, 2023b)**The American Academy of Family Physicians (AAFP)**

The AAFP guidelines recommend various preventive services for children.

For newborns, the AAFP recommends congenital hypothyroidism screening, hearing loss screening, phenylketonuria screening, and sickle cell disease screening. This is closely aligned with USPSTF guidelines (Lin, 2015).

For sexually active adolescent females, the AAFP recommends gonorrhea and chlamydia infection screening (Lin, 2015). The AAFP supports the USPSTF recommendation for syphilis screening as listed above (AAFP, 2016).

For children and adolescents at high risk of infection, the AAFP recommends HIV and Hepatitis B screening (Lin, 2015).

To address and help rectify low-value care practices, Schefft et al. (2019) published on the inception of a series of "do and don't" recommendations in the delivery of healthcare for children and adolescents (Schefft et al., 2019). These recommendations include a suggestion for laboratory-based screening:

- "Don't routinely screen for hyperlipidemia in children and adolescents."

Turner (2018) confirms that the AAFP "generally adheres to USPSTF recommendations" and references several recommendations about screening from the USPSTF and AAP as listed below. The recommendations included below are only those that are within the scope of this medical policy (laboratory-based preventive screening tests):

Screening Recommendations for Children from Birth to 6 Years of Age:

- Dyslipidemia screening by a fasting lipid panel received a grade of "insufficient evidence" by the USPSTF. The AAP recommends "risk-based screening at 2, 4, and 6 years of age (SOR C)."
- Iron deficiency screening by complete blood count received a grade of "insufficient evidence" by the USPSTF. The AAP recommends "screen at 12 months; consider supplements for preterm or exclusively breastfed newborns (SOR C)."
- Lead poisoning screening by measuring lead levels. The USPSTF states that there is "insufficient evidence to recommend screening in children 1 to 5 years of age without increased risk (Grade I)." The AAP recommends "screen[ing] high-risk individuals 6 months to 6 years of age (SOR C)" (Turner, 2018).

National Lipid Association (NLA)

The guidelines list recommendations for "youth" (<20 years old), stating that "Measurement of Lp(a) may be reasonable with:

- Clinically suspected or genetically confirmed FH.
- Individuals with a family history of first-degree relatives with premature ASCVD (<55 y of age in men, 65 y of age in women)
- An unknown cause of ischemic stroke
- A parent or sibling found to have an elevated Lp(a)" (Wilson et al., 2022).

Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

The FDA has approved multiple tests for pediatric preventive screening.

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-

complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

Although the HHS has created the RUSP to provide some standardization for each state's newborn screening programs, the HHS emphasizes that the conditions screened in each program are ultimately decided by the states.

Public Health Service Act (PHS Act)

As per the U.S. Department of Health and Human Services, Section 2713 of the PHS Act "generally requires group health plans and group and individual health insurance issuers that are not grandfathered health plans to provide coverage for recommended preventive services without cost sharing. A complete list of the current recommended preventive services is available at www.healthcare.gov/center/regulations/prevention.html" (HHS, 2020).

National Association of State Boards of Education (NASBE)

The NASBE provides information about state mandates for school health screening (NASBE, 2022).

Please note that individual states may provide specific guidelines and recommendations for pediatric preventive screening.

Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
80061	Lipid panel This panel must include the following: Cholesterol, serum, total (82465) Lipoprotein, direct measurement, high density cholesterol (HDL cholesterol) (83718) Triglycerides (84478)
82247	Bilirubin; total
82248	Bilirubin; direct
82465	Cholesterol, serum or whole blood, total
83020	Hemoglobin fractionation and quantitation; electrophoresis (eg, A2, S, C, and/or F)
83021	Hemoglobin fractionation and quantitation; chromatography (eg, A2, S, C, and/or F)
83655	Lead
83718	Lipoprotein, direct measurement; high density cholesterol (HDL cholesterol)
84439	Thyroxine; free
84443	Thyroid stimulating hormone (TSH)
84478	Triglycerides
85014	Blood count; hematocrit (Hct)
85018	Blood count; hemoglobin (Hgb)
86480	Tuberculosis test, cell mediated immunity antigen response measurement; gamma interferon
86580	Skin test; tuberculosis, intradermal
86850	Antibody screen, RBC, each serum technique
87555	Infectious agent detection by nucleic acid (DNA or RNA); Mycobacteria tuberculosis, direct probe technique
87556	Infectious agent detection by nucleic acid (DNA or RNA); Mycobacteria tuberculosis, amplified probe technique

88720	Bilirubin, total, transcutaneous
0257U	Very long chain acyl-coenzyme A (CoA) dehydrogenase (VLCAD), leukocyte enzyme activity, whole blood Proprietary test: Very-Long Chain Acyl-CoA Dehydrogenase (VLCAD) Enzyme Activity Lab/Manufacturer: Children's Hospital Colorado Laboratory
S3620	Newborn metabolic screening panel, includes test kit, postage and the laboratory tests specified by the state for inclusion in this panel (e.g., galactose; hemoglobin, electrophoresis; hydroxyprogesterone, 17-D; phenylanine (PKU); and thyroxine, total)

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

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Revision History

Revision Date	Summary of Changes
03/06/2024	Reviewed and Updated: Updated the background, guidelines and recommendations, and evidence-based scientific references. Literature review did not necessitate any modifications to coverage criteria.
03/01/2023	Off-Cycle Review: Due to policy reorganization, coverage on screening for Hepatitis B screening (all ages) was moved to G2036-Hepatisis, coverage on screening for chlamydia, gonorrhea, and/or syphilis infection (all ages) was moved to G2157-Diagnostic Testing of Common Sexually Transmitted Infections, and coverage on HIV screening (all ages) was moved to M2116-Human Immunodeficiency Virus. CC8b.ii. previously read: "ii.) 17 – 21 years." Now reads: "ii) For individuals 17 years of age." Coverage of "dyslipidemia using a fasting lipid profile **or** a non-fasting non-HDL-

	C" for individuals 18 years of age and older is addressed in G2050- Cardiovascular Disease Risk Assessment Removed CPT 86689, 86703, 87390, 87391, 87534, 87535, 87536, 87537, 87538, 87539, 87806, S3645, 86704, 86707, 86705, 86706, 87320, 87340, 87341, 87516, 87517 (Removed HIV coverage criteria with reference to policy M2116 and Hep B coverage criteria with reference to policy G2036) Coding Enhancement: Added CPT 83020, 83021, removed 85660
12/07/2022	Reviewed and Updated: Updated the background, guidelines and recommendations, and evidence-based scientific references. Literature review did not necessitate any modifications to coverage criteria. The following edits were made for clarity: All CC except CC10 were edited for clarity and consistency. CC12 was removed as a CC and is now a note in the Policy Description to see AHS-G2006 for guidance on Hemoglobin A1c screening. CC5.b., CC6.b., CC7, CC8.a., and CC9 all contained a list of high risk situations- these have all been moved into Notes, resulting in Notes 1-5. Removed CPT 86592, 86593, 86631, 86632, 86780, 87110, 87270, 87320, 87490, 87491, 87590, 87591, 87810, 87850
03/09/2022	Annual review: Updated background, guidelines, and evidence-based scientific references. Literature review did not necessitate any modification to coverage criteria. The following changes were made for clarity: CC5a: remove all "children": Ages 12 months to 2 years and CC5b: remove "children": Ages 6 months to 6 years who are at increased risk for lead exposure, as defined by the AAP (poor, those who are recent immigrants, those in older, poorly maintained housing, those who had a sibling or playmate with an elevated blood lead concentration, those who have parents exposed to lead at work, or those who had lived in or visited a structure that might contain deteriorated, damaged, or recently remodeled lead-painted surfaces). CC7: added "with an individual who": Tuberculosis screening MEETS COVERAGE CRITERIA for children age 1 month and older who are at increased risk: born in a country other than the U.S., Canada, Australia, New Zealand, or Western Europe, traveled (had contact with resident populations) for longer than 1 week to a country with high risk for tuberculosis, has a family member or contact with an individual who had tuberculosis or a positive tuberculin skin test, or is infected with HIV. CPT Changes: Added 0257U
03/03/2021	Annual review: Updated background, guidelines, and evidence-based scientific references. Literature review did not necessitate any modification to coverage criteria.
03/10/2020	Annual review: Updated background, guidelines, and evidence-based scientific references. The following changes were made for uniformity between policies: • CC concerning screening for diabetes using Hemoglobin A1c now refers to policy AHS-G2006 Hemoglobin A1c. • CC concerning screening for HIV was reworded, based on guidelines from Bright Futures, CDC, and USPSTF to be stated identically to the CC in G2009: "Screening for HIV infection MEETS COVERAGE CRITERIA in adolescents and adults, ages 11 to 65 years."

03/01/2019	Annual review: Updated background, guidelines, and evidence-based scientific references. Literature review did not necessitate any modification to coverage criteria. Updated table to include 86850. Removed 83037 and 82962 (out of scope).
5/14/2018	Off cycle review: Added CPT codes 83036 and 84439 as PA Not Required.
03/16/2018	Annual Review: Literature review did not necessitate any modification to coverage criteria. Removed CPT 83987, 84030 and 84436.
01/16/2018	Off cycle review: Removed CPT codes 88141, 88142, 88143, 88147, 88148, 88150, 88152, 88153, 88160, 88161, 88162, 88164, 88165, 88166, 88167, P3000, P3001, and Q0091, added CPT codes 82247, 82248, 82962, 86850, 87555 and 87556 as no PA required
01/1/2018	Off cycle review: Removed CPT code 87515 as code inactive effective 1/1/2018
12/14/2017	Off-cycle review to address only ACA parameters as identified as a gap with prior policy. CC changes: added newborn screening, bilirubin, and tuberculosis screening; expanded age range for anemia, lead, and dyslipidemia; updated age range and risk factors for HIV. Changed name from "Children and Adolescent" to "Pediatric": CC1 addition to align with ACA which requires newborn screening guidelines to follow the federal Recommended Uniform Screening Panel guidelines.link; prior CC3 removed as included in new CC1;-prior CC4 removed as included in new CC5;-CC5 reworded per ACA screening recommendation as per Bright Futures/AAP link;-CC6 reworded per ACA screening recommendation as per Bright Futures/AAP;-New CC7 added per ACA screening recommendation as per Bright Futures/AAP link;-Prior CC 7- reworded per ACA screening recommendation as per Bright Futures/AAP link;- New CC9 consolidates prior CC9, CC10 and CC14; New CC14 consolidates prior CC12 and CC13
01/16/2017	Off cycle review. References updated.
12/12/2016	Annual review completed. Added coverage criteria related to screening for anemia at approximately 12 months of age (AAP, 2016). Confirmed age range for policy to be for individuals prior to 19th birthday.
11/16/2015	Initial presentation

Prenatal Screening (Nongenetic)

Policy Number: AHS – G2035 – Prenatal Screening (Nongenetic)	Prior Policy Name and Number, as applicable: AHS – G2035 – Prenatal Screening
Initial Presentation Date: 06/16/2015 Effective Date: 02/01/2025	

Policy Description

Prenatal screening encompasses any testing done to determine the health status of the pregnant individual and/or fetus. Biochemical prenatal screening encompasses screening for infectious diseases and conditions that may complicate the pregnancy. Screening refers to testing of asymptomatic or healthy individuals to search for a condition that may affect the pregnancy or individual, whereas diagnostic testing is used to either confirm or refute true abnormalities in an individual (Grant & Mohide, 1982; Lockwood & Magriples, 2024).

For guidance on thyroid screening in pregnant individuals, please see AHS-G2045-Thyroid Disease Testing. For guidance on fetal aneuploidy screening, please see AHS-G2055-Prenatal Testing for Fetal Aneuploidy. For guidance on screening for Zika virus infection in pregnant individuals, please see AHS-G2158-Testing for Vector-Borne Infections.

Related Policies

Policy Number	Policy Title
AHS-G2036	Hepatitis Testing
AHS-G2045	Thyroid Disease Testing
AHS-G2055	Prenatal Testing for Fetal Aneuploidy
AHS-G2157	Diagnostic Testing of Common Sexually Transmitted Infections
AHS-G2158	Testing for Vector-Borne Infections.
AHS-G2159	β-Hemolytic Streptococcus Testing
AHS-M2116	Human Immunodeficiency Virus (HIV)
AHS-M2141	Testing of Homocysteine Metabolism-Related Conditions
AHS-M2179	Prenatal Screening (Genetic)

Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) The following routine prenatal screening **MEETS COVERAGE CRITERIA** for all pregnant individuals:
   a) Antigen/antibody combination assay screening for HIV infection.
   b) Screening for *Chlamydia trachomatis* infection.
   c) Screening for *Neisseria gonorrhoeae* infection.
   d) Triple panel screening (HBsAg, anti-HBs, total anti-HBc) for hepatitis B.
   e) Screening for syphilis.
   f) Antibody screening for hepatitis C.
   g) Screening for type 2 diabetes at the first prenatal visit.
   h) Screening for gestational diabetes during gestational weeks 24 – 28 and at the first prenatal visit if risk factors are present.
   i) Determination of blood type, Rh(D) status, and antibody status during the first prenatal visit, and repeated Rh (D) antibody testing for all unsensitized Rh (D)-negative individuals at 24 to 28 weeks' gestation, unless the biological father is known to be Rh (D)-negative.
   j) Screening for anemia with a CBC or hemoglobin and hematocrit with mean corpuscular volume.

k) Screening for Group B streptococcal disease (once per pregnancy; recommended during gestational weeks 36 to 37).

l) Urinalysis and urine culture.

m) Rubella antibody testing.

n) Testing for varicella immunity.

o) Screening for tuberculosis in pregnant individuals deemed to be at high risk for TB.

2) For pregnant individuals who are less than 25 years of age or who are at a continued high risk of infection (e.g., individual has: new or multiple sex partners, a history of sexually transmitted infections, past or current injection drug use), third trimester re-screening of *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, syphilis, and/or HIV infections **MEETS COVERAGE CRITERIA**.

3) For individuals who are pregnant with singleton or twin pregnancies and who are presenting in the ambulatory setting with signs or symptoms of preterm labor, a fetal fibronectin (FFN) assay **MEETS COVERAGE CRITERIA**.

a)

4) For individuals with a normal pregnancy without complications, human chorionic gonadotropin (hCG) hormone testing **DOES NOT MEET COVERAGE CRITERIA**.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

5) As a technique of risk assessment for preterm labor or delivery, serial monitoring of salivary estriol levels **DOES NOT MEET COVERAGE CRITERIA**.

Table of Terminology

Term	Definition
ACMG	American College of Medical Genetics and
ACOG	American College of Obstetricians and
ADA	American Diabetes Association
CDC	Centers for Disease Control and Prevention
EIA	Enzyme immunoassay
ELISA	Enzyme linked immunosorbent assay
FFN	Fetal fibronectin
GBS	Group B streptococcal disease
GDM	Gestational diabetes mellitus

HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HDFN	Hemolytic disease of the fetus and newborn
HIV	Human immunodeficiency virus
HRSA	Health Resources & Services Administration
HSV	Herpes simplex virus
PAH	Phenylalanine hydroxylase
PITC	Provider-initiated HIV testing and counselling
RBC	Red blood cells
RhD	Rh blood group D antigen
STI	Sexually transmitted infection
TB	Tuberculosis
TMRC	Transfusion Medicine Resource Committee
VA/DoD	Veterans Affairs/Department of Defense
WHO	World Health Organization

Scientific Background

Prenatal screening is a part of overall prenatal care to promote optimal care of both mother and baby and allows for assessment and monitoring of the fetus for the presence of congenital defects or disease. Various professional medical organizations provide guidelines for prenatal screening. "Screening is an offer on the initiative of the health system or society, rather than a medical intervention in answer to a patient's complaint or health problem. Screening aims at obtaining population health gains through early detection that enables prevention or treatment" (de Jong et al., 2015).

Routine prenatal screening may include several laboratory tests, such as hematocrit or hemoglobin testing to check for anemia and possible thalassemia, pending further diagnostic testing. Blood typing and antibody screening can be performed to prevent possible alloimmunization or hemolytic diseases and glucose testing can screen for possible gestational diabetes mellitus. Screening for asymptomatic bacteriuria and proteinuria is recommended as well as screening for infectious disorders, such as HIV, syphilis, chlamydia, and gonorrhea (Lockwood & Magriples, 2024).

Red blood cell antigen discrepancy between a mother and fetus may also occur during pregnancy. This is known as hemolytic disease of the fetus and newborn (HDFN), and causes maternal antibodies to destroy the red blood cells of the neonate or fetus (Calhoun, 2024). Alloimmunization is the immune response which occurs in the mother due to foreign antigens after exposure to genetically foreign cells, occurring almost exclusively in mothers with type O blood. However, while ABO blood type incompatibility is identified in almost 15% of pregnancies, HDFN is only identified in approximately 4% of pregnancies (Calhoun, 2024). Another important inherited

antigen sometimes found on the surface of red blood cells is known as the Rhesus (Rh)D antigen. During pregnancy and delivery, individuals who are RhD negative may be exposed to RhD positive fetal cells, which can lead to the development of anti-RhD antibodies. This exposure typically happens during delivery and affects subsequent pregnancies; infants with RhD incompatibility tend to experience a more severe form of HDFN than those with ABO incompatibility (Calhoun, 2024). The clinical presentation of HDFN may be mild (such as hyperbilirubinemia with mild to moderate anemia) to severe and life-threatening anemia (such as hydrops fetalis) (Calhoun, 2024). Less severely affected infants may develop hyperbilirubinemia within the first day of life; infants with RhD HDFN may also present with symptomatic anemia requiring a blood transfusion. In more severe cases, infants with severe life-threatening anemia, such as hydrops fetalis, may exhibit shock at delivery requiring an emergent blood transfusion (Calhoun, 2024).

The administration of anti-D immune globulin has been able to dramatically reduce, but not eliminate, the number of RhD alloimmunization cases. "Anti-D immune globulin is manufactured from pooled plasma selected for high titers of IgG antibodies to D-positive erythrocytes" (Moise Jr, 2024). Before the development of this anti-D immune globulin, it has been reported that 16% of pregnant RhD-negative individuals with two deliveries of RhD-positive ABO-compatible infants became alloimmunized. However, this rate falls to 1-2% with routine postpartum administration of a single dose of anti-D immune globulin. An additional administration in the third trimester of pregnancy further reduces the incidents of alloimmunization to 0.1-0.3% (Moise Jr, 2024).

Fetal fibronectin (FFN) is a protein made during pregnancy that is found between the lining of the uterus and the amniotic sac, at the decidual-chorionic interface. FFN is often described as being the glue that holds the amniotic sac to the uterine lining (URMC, 2024). Disruption of the decidual-chorionic interface releases FFN into cervicovaginal secretions, allowing FFN to be used as a marker for predicting spontaneous preterm birth in individuals with singleton and twin gestations (Lockwood et al., 1991). A meta-analysis of 11 studies found that under 10% of pregnant people with low FFN (<10 ng/mL) delivered before 34 weeks, while 37-67% of pregnant people with high FFN (>200 ng/mL) delivered before 34 weeks (Blackwell et al., 2017). The negative predictive value of FFN in predicting preterm birth is 99.5% within seven days, 99.2% within 14 days, and 84.5% before 37 weeks (Peaceman et al., 1997). FFN is also useful in singleton and twin pregnancies, as multiple pregnancy is a risk factor for preterm birth. For singleton and multiple pregnancies, FFN has a negative predictive value of 100%, sensitivity of 100% for delivery within 10 days, but a positive predictive value of 10% and a specificity of 64% (Cornelissen et al., 2020).

Human chorionic gonadotropin (hCG) is a biomarker in the glycoprotein hormone family. Other hormones in this family include luteinizing hormone (LH), follicle-stimulating hormone (FSH), and thyroid stimulating hormone. Human chorionic gonadotropin in pregnancy serves as an important biomarker for the detection of pregnancy-related disorders and hCG is also measured in some prenatal tests for Down syndrome. Low levels of hCG are associated with pregnancy loss and preeclampsia, while high levels can be associated with Down syndrome pregnancies (Richard Alan Harvey, 2023). A qualitative hCG test may be used to screen for pregnancy and gives a simple positive or negative result. A quantitative hCG measurement is used to assess pregnancy viability and screen for disorders. Quantitative hCG tests measures the exact amount of hCG in blood; for example, during 10-12 weeks of gestation, hCG levels are expected to approximately double every 24-48 hours, such that abnormal measurement results for hCG may indicate issues with the pregnancy (AACC, 2023).

Clinical Utility and Validity

Education and counseling are a key factor in prenatal screening and diagnostic tests. Yesilcinar and Guvenc (2021) found that a proactive intervention approach decreased anxiety and decisional conflict in the pregnant individual and increased attitudes towards the tests, having a positive effect on the pregnant individual's knowledge level and decision satisfaction. This allowed the individual to make more informed decisions, such as opting to have screening and diagnostic testing performed (Yesilcinar & Guvenc, 2021).

Implementation of prenatal screening tests can positively affect pregnancies and pregnancy outcomes. The Centers for Disease Control and Prevention (CDC) reports that implementation of the 1996 guidelines concerning Group B Streptococcus (GBS) had a profound effect. Prior to screening and widespread use of intrapartum antibiotics, invasive neonatal GBS occurred in two to three cases per 1,000 live births; however, after prenatal screening implementation, the rate declined to 0.5 cases per 1,000 live births in 1999 (Schrag et al., 2002). The CDC also reports from a multi-year study that screening for syphilis in all pregnant individuals at the first prenatal visit (and then rescreening in third trimester for individuals at risk) is very important in preventing congenital syphilis, which can cause spontaneous abortion, stillbirth, and early infant death. They show that 88.2% of cases of congenital syphilis was avoided when proper screening was applied; moreover, 30.9% of the cases of congenital syphilis that did occur happened when the mother did not receive proper prenatal care (≥45 days before delivery) (Slutsker et al., 2018).

Guidelines and Recommendations

American College of Obstetricians and Gynecologists (ACOG)

The American College of Obstetricians and Gynecologists has several practice guidelines related to prenatal care as well as both pre-conception and prenatal testing. ACOG recommendations and guidelines include the following:

- **Vitamin D Screening:** Concerning vitamin D screening, "there is insufficient evidence to support a recommendation for screening all pregnant [individuals] for vitamin D deficiency. For pregnant [individuals] thought to be at increased risk of vitamin D deficiency, maternal serum 25-hydroxyvitamin D levels can be considered and should be interpreted in the context of the individual clinical circumstance" (ACOG, 2011). This was reaffirmed in 2024.
- **Lead Screening:** Concerning lead screening, ACOG recommends "evaluating risk factors for exposure as part of a comprehensive health risk assessment and perform blood lead testing if a single risk factor is identified. Assessment of lead exposure should take place at the earliest contact with the pregnant patient" (ACOG, 2012). This position was reaffirmed in 2023.
- **Depression and Anxiety:** ACOG "recommends screening patients at least once during the perinatal period for depression and anxiety, and, if screening in pregnancy, it should be done again postpartum." Further, ACOG "recommends a full assessment of physical, social, and psychological well-being within a comprehensive postpartum visit that occurs no later than 12 weeks after birth" (ACOG, 2024).
- ***Listeria monocytogenes:*** Concerning testing for *Listeria monocytogenes*, "No testing, including blood and stool cultures, or treatment is indicated for an asymptomatic pregnant [individual] who reports consumption of a product that was recalled or implicated during an outbreak of listeria contamination. An asymptomatic patient should be instructed to return if she develops symptoms of listeriosis within 2 months of eating the recalled or implicated product" (ACOG, 2014). If an exposed pregnant individual shows signs and symptoms consistent with infection, then blood culture testing is the standard of care. Stool culture testing is not recommended since it has not been validated as a screening tool (ACOG, 2014). This position was reaffirmed in 2023.
- **HIV:** Concerning HIV, ACOG recommends that all individuals should be tested for HIV with the right to refuse testing. "Human immunodeficiency virus testing using the opt-out approach, which is currently permitted in every jurisdiction in the United States, should be a routine component of care for [individuals] during prepregnancy and as early in pregnancy as possible. Repeat HIV testing in the third trimester, preferably before 36 weeks of gestation, is recommended for

pregnant [individuals] with initial negative HIV antibody tests who are known to be at high risk of acquiring HIV infection; who are receiving care in facilities that have an HIV incidence in pregnant [individuals] of at least 1 per 1,000 per year; who are incarcerated; who reside in jurisdictions with elevated HIV incidence; or who have signs and symptoms consistent with acute HIV infection (e.g., fever, lymphadenopathy, skin rash, myalgias, arthralgias, headache, oral ulcers, leukopenia, thrombocytopenia, or transaminase elevation). Rapid screening during labor and delivery or during the immediate postpartum period using the opt-out approach should be done for [individuals] who were not tested earlier in pregnancy or whose HIV status is otherwise unknown. Results should be available 24 hours a day and within 1 hour" (ACOG, 2018). This position was reaffirmed in 2024.
    - For pregnant individuals who test positive for HIV, "Additional laboratory work, including $CD4^+$ count; HIV viral load; testing for antiretroviral resistance; hepatitis C virus antibody; hepatitis B surface antigen and viral load; and hepatitis A using antibody testing for immunoglobulin G for [individuals] who have hepatitis B virus infection and who have not already received the hepatitis A virus vaccine series; complete blood count with platelet count; and baseline chemistries with comprehensive metabolic testing, will be useful before prescribing antiretroviral therapy" (ACOG, 2018). This opinion was reaffirmed in 2024.
- **Prevention of Rh D Alloimmunization:** Concerning the prevention of Rh D alloimmunization, ACOG has published the guidelines supporting the administration of anti-D immune globulin to individuals in various scenarios. However, these guidelines do not mention the use of cell-free fetal DNA for fetal RHD testing to determine if anti-D immune globulin is needed (ACOG, 2017).
- **Group B Streptococcal (GBS) Disease:** "all pregnant [individuals] should undergo antepartum screening for GBS at 36 0/7–37 6/7 weeks of gestation, unless intrapartum antibiotic prophylaxis for GBS is indicated because of GBS bacteriuria during the pregnancy or because of a history of a previous GBS-infected newborn. This new recommended timing for screening provides a 5-week window for valid culture results that includes births that occur up to a gestational age of at least 41 0/7 weeks" (ACOG, 2020). This position was reaffirmed in 2022.
- **Lab Tests:** ACOG lists the following lab tests to be performed early in pregnancy: complete blood count (CBC), blood type and Rh factor, urinalysis, urine culture, rubella, hepatitis B, hepatitis C, HIV, sexually transmitted infection (STI) testing, and tuberculosis (ACOG, 2024). ACOG lists the following lab tests to be performed later in pregnancy: glucose screening test and Group B streptococcus (GBS) screening (ACOG, 2024).

United States Preventive Services Task Force (USPSTF)

The United States Preventive Services Task Force (USPSTF) recommends the following testing for pregnant individuals:

- Screening for gestational diabetes in asymptomatic pregnant individuals at ≥24 weeks of gestation (Grade B) (USPSTF, 2021b).
- Screening for hepatitis B virus (HBV) infection at the first prenatal visit (Grade A) (USPSTF, 2019d).
- Screening for asymptomatic bacteriuria with urine culture is recommended in pregnant persons (Grade B) (USPSTF, 2019a).
- Screening for HIV is recommended in all pregnant persons, including those in labor or whose HIV status is unknown at delivery (Grade A) (USPSTF, 2019e).
- Rh (D) blood typing and antibody testing for all pregnant individuals during their first visit for pregnancy-related care (Grade A) (USPSTF, 2005).
- Repeated Rh (D) antibody testing for all unsensitized Rh (D)-negative individuals at 24-28 weeks' gestation, unless the biological father is known to be Rh (D)-negative (Grade B) (USPSTF, 2005).
- Screening early for syphilis infection in all pregnant individuals (Grade A) (USPSTF, 2018).

Additional recommendations from the USPSTF that may be relevant during pregnancy include:

- The USPSTF recommends screening for chlamydia in all sexually active [individuals] 24 years or younger and in [individuals] 25 years or older who are at increased risk for infection (Grade B)(USPSTF, 2021a).
- The USPSTF recommends screening for gonorrhea in all sexually active [individuals] 24 years or younger and in [individuals] 25 years or older who are at increased risk for infection (Grade B) (USPSTF, 2021a).
- The USPSTF recommends that clinicians provide or refer pregnant and postpartum persons who are at increased risk of perinatal depression to counseling interventions (Grade B) (USPSTF, 2019b).

Screening for hepatitis C virus (HCV) infection is recommended in all adults aged 18 to 79 years (Grade B) (Graham & Trooskin, 2020).

Concerning screening adults for drug use, Krist et al. (2020) state that "the USPSTF recommends screening by asking questions about unhealthy drug use in adults ages 18 years or older. Screening should be implemented when services for accurate diagnosis, effective treatment, and appropriate care can be offered or referred. (Screening refers to asking questions about unhealthy drug use, not testing biological specimens.)" The USPSTF also states that "this new evidence supports the current recommendation that primary care clinicians offer screening to adults 18 years or

older, including those who are pregnant or postpartum, when services for accurate diagnosis, effective treatment, and appropriate care can be offered or referred" (Krist et al., 2020).

However, the USPSTF recommends against the following tests during pregnancy:

- Screening for bacterial vaginosis in pregnant individuals who are not at risk for preterm delivery (grade D); further, current evidence is insufficient for screening pregnant persons who are at increased risk for preterm delivery (USPSTF, 2020).
- Serological screening for herpes simplex virus (HSV) in asymptomatic pregnant individuals (USPSTF, 2023).
- Screening for elevated blood lead levels in asymptomatic pregnant individuals has been given an I recommendation as current evidence is insufficient to determine if this testing is beneficial or not (USPSTF, 2019c).
- "The USPSTF concludes that the current evidence is insufficient to assess the balance of benefits and harms of screening for iron deficiency anemia in pregnant [individuals] to prevent adverse maternal health and birth outcomes" (Siu, 2015).

American Diabetes Association (ADA)

The American Diabetes Association in the 2023 *Standards of Medical Care in Diabetes* make the following recommendations (ADA, 2023):

- "Starting at puberty and continuing in all [individuals] with diabetes and reproductive potential, preconception counseling should be incorporated into routine diabetes care. [Grade] **A**
- Preconception counseling should address the importance of achieving glucose levels as close to normal as is safely possible, ideally A1C <6.5% (48 mmol/mol), to reduce the risk of congenital anomalies, preeclampsia, macrosomia, preterm birth, and other complications. [Grade] **A**
- Individuals with preexisting diabetes who are planning a pregnancy should ideally begin receiving care in preconception at a multidisciplinary clinic in including an endocrinologist, maternal-fetal medicine specialist, registered dietitian nutritionist, and diabetes care and education specialist, when available. [Grade] **B**
- In addition to focused attention on achieving glycemic targets, standard preconception care should be augmented with extra focus on nutrition, diabetes education, and screening for diabetes comorbidities and complications. [Grade] B
- Individuals with preexisting type 1 or type 2 diabetes who are planning pregnancy or who have become pregnant should be counseled on the risk of development and/or progression of diabetic retinopathy. Dilated eye examinations should occur ideally before pregnancy or in the first trimester, and then patients should be monitored every trimester and for 1 year postpartum as

indicated by the degree of retinopathy and as recommended by the eye care provider. [Grade] **B**

- Screen individuals with a recent history of gestational diabetes mellitus at 4–12 weeks postpartum, using the 75-g oral glucose tolerance test and clinically appropriate nonpregnancy diagnostic criteria. [Grade] **B**
- Individuals with a history of gestational diabetes mellitus should have lifelong screening for the development of type 2 diabetes or prediabetes every 1–3 years. [Grade] **B**
- Individuals with a history of gestational diabetes mellitus found to have prediabetes should receive intensive lifestyle interventions and/or metformin to prevent diabetes. [Grade] **A**
- Individuals with a history of gestational diabetes mellitus should seek preconception screening for diabetes and preconception care to identify and treat hyperglycemia and prevent congenital malformations. [Grade] **E**"

Centers for Disease Control and Prevention (CDC)

The Centers for Disease Control and Prevention (CDC) recommends (CDC, 2024d):

Disease	**Recommendations for Pregnant Individuals**
Chlamydia	All pregnant individuals under 25 years of age Pregnant individuals 25 years of age and older if at increased risk* Retest during the 3rd trimester for individuals under 25 years of age or at risk Pregnant individuals with chlamydial infection should have a test of cure 4 weeks after treatment and be retested within 3 months
Gonorrhea	All pregnant individuals under 25 years of age, and those 25 and older if at increased risk* Retest during the 3rd trimester for individuals under 25 years of age or at risk Pregnant individuals with gonorrhea should be retested within 3 months
Syphilis	All pregnant individuals at the first prenatal visit Retest at 28 weeks gestation and at delivery if at increased risk due to geography or personal risk (substance use, STIs during pregnancy, multiple partners, a new partner, partner with STIs
Herpes†	Routine HSV-2 serologic screening among asymptomatic pregnant individuals is not recommended. However, type-specific serologic tests might be useful for identifying pregnant individuals at risk for HSV infection and guiding counseling regarding the risk for acquiring genital herpes during pregnancy.

HIV	All pregnant individuals should be screened at first prenatal visit (opt-out) Retest in the 3rd trimester if at increased risk (people who use drugs, have STIs during pregnancy, have multiple sex partners during pregnancy, have a new sex partner during pregnancy, live in areas with high HIV prevalence, or have partners with HIV) Rapid testing should be performed at delivery if not previously screened during pregnancy
HPV, Cervical Cancer	Pregnant individuals should be screened at same intervals as nonpregnant individuals
Hepatitis B Screening	Test for HBsAg at first prenatal visit of each pregnancy regardless of prior testing; retest at delivery if at increased risk
Hepatitis C Screening	Pregnant individuals should be screened for hepatitis C except in settings where the hepatitis C infection (HCV) positivity is < 0.1%

"* Per USPSTF, sexually active [individuals] 25 years or older are at increased risk for chlamydial and gonococcal infections if they have a new partner, more than one sex partner, a sex partner with concurrent partners, or a sex partner who has an STI; practice inconsistent condom use when not in a mutually monogamous relationship; have a previous or coexisting STI; have a history of exchanging sex for money or drugs; or have a history of incarceration.

† Type-specific HSV-2 serologic assays for diagnosing HSV-2 are useful in the following scenarios: recurrent or atypical genital symptoms or lesions with a negative HSV PCR or culture result, clinical diagnosis of genital herpes without laboratory confirmation, and a patient's partner has genital herpes. HSV-2 serologic screening among the general population is not recommended. Patients who are at higher risk for infection (e.g., those presenting for an STI evaluation, especially for persons with ≥10 lifetime sex partners, and persons with HIV infection) might need to be assessed for a history of genital herpes symptoms, followed by type-specific HSV serologic assays to diagnose genital herpes for those with genital symptoms" (CDC, 2024d).

- "Everyone who is pregnant should be tested for syphilis, HIV, hepatitis B, and hepatitis C starting early in pregnancy. Repeat testing may be needed" (CDC, 2024b).
- Pregnant people at risk should also be tested for chlamydia and gonorrhea starting early in pregnancy. Repeat testing may be needed in some cases" (CDC, 2024b).
- "A second test during the third trimester, preferably at <36 weeks' gestation, should be considered and is recommended for [individuals] who are at high risk

for acquiring HIV infection, [individuals] who receive health care in jurisdictions with high rates of HIV, and [individuals] examined in clinical settings in which HIV incidence is ≥1 per 1,000 [individuals] screened per year" (CDC, 2021e).

- "Providers should use a laboratory-based antigen/antibody (Ag/Ab) combination assay as the first test for HIV, unless persons are unlikely to follow up with a provider to receive their HIV test results; in those cases screening with a rapid POC test can be useful" (CDC, 2021e).
- "Regardless of whether they have been previously tested or vaccinated, all pregnant [individuals] should be tested for HBsAg at the first prenatal visit and again at delivery if at high risk for HBV infection (see STI Detection Among Special Populations). Pregnant [individuals] at risk for HBV infection and without documentation of a complete hepatitis B vaccine series should receive hepatitis B vaccination" (CDC, 2021c).
- Recommendation for HBV screening in "All pregnant persons during each pregnancy, preferably in the first trimester, regardless of vaccination status or history of testing" (CDC, 2023).
- "Pregnant persons with a history of appropriately timed triple panel screening and without subsequent risk for exposure to HBV (i.e., no new HBV exposures since triple panel screening) only need HBsAg screening. Testing pregnant persons known to be chronically infected or immune enables documentation of the HBsAg test result during that pregnancy to ensure timely prophylaxis for exposed infants" (CDC, 2023).
- "Using the triple panel (HBsAg, anti-HBs, and total anti-HBc) is recommended for initial screening because it can help identify persons who have an active HBV infection and could be linked to care, have resolved infection and might be susceptible to reactivation (e.g., immunosuppressed persons), are susceptible and need vaccination, or are vaccinated. When someone receives triple panel screening, any future periodic testing can use tests as appropriate (e.g., only HBsAg and anti-HBc if the patient is unvaccinated)" (CDC, 2023).
- "[individuals] aged <25 years and those at increased risk for chlamydia (i.e., those who have a new sex partner, more than one sex partner, a sex partner with concurrent partners, or a sex partner who has an STI) should be screened at the first prenatal visit and rescreened during the third trimester to prevent maternal postnatal complications and chlamydial infection in the infant" (CDC, 2021b).
- "Annual screening for *N. gonorrhoeae* infection is recommended for all sexually active [individuals] aged <25 years and for older [individuals] at increased risk for infection (e.g., those aged ≥25 years who have a new sex partner, more than one sex partner, a sex partner with concurrent partners, or a sex partner who has an STI . . . [All individuals] who have been treated for gonorrhea should be retested 3

months after treatment regardless of whether they believe their sex partners were treated" (CDC, 2022).

- "CDC recommends hepatitis C screening . . . all [individuals] during each pregnancy, except in settings where the prevalence of HCV infection is <0.1%" (CDC, 2021d).
- Zika virus recommendations for asymptomatic pregnant patients:
  - "Lived in or traveled to the United States and its territories during pregnancy: Since no confirmed cases of Zika virus disease have been detected in the United States and its territories since 2018, routine Zika virus testing is not recommended."
  - "Traveled to an area with an active CDC Zika Travel Health Notice during pregnancy: NAAT testing may be considered up to 12 weeks after travel."
  - "Traveled to an area with current or past Zika virus transmission outside the US and its territories during pregnancy: Routine testing is not recommended. If the decision is made to test, NAAT testing can be done up to 12 weeks after travel"- (CDC, 2024a).
- Zika virus recommendations for symptomatic pregnant patients:
  - "Lived in or traveled to an area with an active CDC Zika Travel Health Notice during pregnancy OR had sex during pregnancy with someone living in or with recent travel to an area with an active CDC Zika Travel Health Notice:
    - Specimens should be collected as soon as possible after onset of symptoms up to 12 weeks after symptom onset.
    - Perform dengue and Zika virus NAAT and IgM testing on a serum specimen and Zika virus NAAT on a urine specimen.
    - If Zika NAAT is positive and the Zika IgM is negative, repeat NAAT test on newly extracted RNA from same specimen to rule out false-positive results.
    - If both dengue and Zika virus NAATs are negative but either IgM antibody test is positive, confirmatory PRNTs should be performed against dengue, Zika, and other flaviviruses endemic to the region where exposure occurred.
  - Lived in or traveled to an area with current or past Zika virus transmission during pregnancy:
    - Specimens should be collected as soon as possible after onset of symptoms up to 12 weeks after symptom onset.
    - Perform dengue and Zika virus NAAT testing on a serum specimen and Zika virus NAAT on a urine specimen.
    - If Zika NAAT is positive, repeat test on newly extracted RNA from same specimen to rule out false-positive results.
    - Perform IgM testing for dengue only.

    - If dengue NAAT or IgM test is positive, this provides adequate evidence of dengue infection, and no further testing is indicated.
  - Had sex during pregnancy with someone living in or with recent travel to an area with current or past Zika virus transmission:
    - Specimens should be collected as soon as possible after onset of symptoms up to 12 weeks after symptom onset.
    - Only Zika NAAT should be performed.
    - If Zika NAAT is positive, repeat test on newly extracted RNA from same specimen to rule out false-positive results" (CDC, 2024a).
- Zika virus recommendations for pregnant patients having a fetus with prenatal ultrasound findings consistent with congenital Zika virus infection:
  - "Lived in or traveled during pregnancy to an area with an active CDC Zika Travel Health Notice or current or past Zika virus transmission OR had sex during pregnancy with someone living in or with recent travel to an area with an active CDC Zika Travel Health Notice or current or past Zika virus transmission:
    - Zika virus NAAT and IgM testing should be performed on pregnant person's serum and NAAT on pregnant person's urine.
    - If the Zika virus NAATs are negative and the IgM is positive, confirmatory PRNTs should be performed against Zika and dengue.
    - If amniocentesis is being performed as part of clinical care, Zika virus NAAT testing of amniocentesis specimens should also be performed and results interpreted within the context of the limitations of amniotic fluid testing.
    - Testing of placental and fetal tissues may also be considered" (CDC, 2024a).
  - "Evidence does not support routine screening for BV among asymptomatic pregnant [individuals]

at high risk for preterm delivery (159). Symptomatic [individuals] should be evaluated and treated (see Bacterial Vaginosis). Evidence does not support routine screening for Trichomonas vaginalis among asymptomatic pregnant [individuals]. [Individuals] who report symptoms should be evaluated and treated (see Trichomoniasis). In addition, evidence does not support routine HSV-2 serologic screening among asymptomatic pregnant [individuals]. However, type-specific serologic tests might be useful for identifying pregnant [individuals] at risk for HSV-2 infection and for guiding counseling regarding the risk for acquiring genital herpes during pregnancy. Routine serial cultures for HSV are not indicated for [individuals] in the third trimester who have a history of recurrent genital herpes"(CDC, 2021a).

- "Prenatal screening for some infections (HIV, syphilis, hepatitis B virus, and hepatitis C virus) is recommended for all pregnant [individuals]. Screening for other infections (chlamydia, gonorrhea, and TB) is recommended for some [individuals] at risk for infection" (CDC, 2024c).

American College of Medical Genetics and Genomics (ACMG)

In 2014, the ACMG released guidelines concerning the diagnosis and management of phenylalanine hydroxylase (PAH) deficiency. They recommend PAH testing be part of newborn screening and that "quantitative blood amino acids testing should be performed for diagnostic testing following a positive newborn screen of PAH deficiency. Additional testing is needed to define the cause of elevated PHE and should include analysis of pterin metabolism; PAH genotypic is indicated for improved therapy planning" (Vockley et al., 2014).

World Health Organization (WHO)

In 2016, the WHO released their publication titled, *WHO recommendations on antenatal care for a positive pregnancy experience*, which had the following recommendations (WHO, 2016):

- Anemia (Context-specific recommendation)—"Full blood count testing is the recommended method for diagnosing anaemia in pregnancy."
- Asymptomatic bacteriuria (Context-specific recommendation)—"Midstream urine culture is the recommended method for diagnosing asymptomatic bacteriuria (ASB) in pregnancy. In settings where urine culture is not available, on-site midstream urine Gram-staining is recommended over the use of dipstick tests as the method for diagnosing ASB in pregnancy."
- Gestational diabetes mellitus (Recommended)—"Hyperglycaemia first detected at any time during pregnancy should be classified as either gestational diabetes mellitus (GDM) or diabetes mellitus in pregnancy, according to WHO criteria."
- HIV and syphilis (Recommended)—"In high-prevalence settings, provider-initiated HIV testing and counselling (PITC) for HIV should be considered a routine component of the package of care for pregnant [individuals] in all antenatal care settings. In low-prevalence settings, PITC can be considered for pregnant [individuals] in antenatal care settings as a key component of the effort to eliminate mother-to-child transmission of HIV, and to integrate HIV testing with syphilis, viral or other key tests, as relevant to the setting, and to strengthen the underlying maternal and child health systems."
- Tuberculosis (Context-specific recommendation)—"In settings where the tuberculosis (TB) prevalence in the general population is 100/100 000 population

or higher, systematic screening for active TB should be considered for pregnant [individuals] as part of antenatal care" (WHO, 2016).

Department of Veterans Affairs/Department of Defense (VA/DoD)

In the 4th edition of the VA/DoD *Clinical Practice Guideline for the Management of Pregnancy* (VA & DOD, 2023), they list the following lab tests as routine for all pregnancies in the first prenatal visit: HIV, CBC, ABO Rh blood typing, Antibody screen, gonorrhea, chlamydia, hepatitis C antibody, syphilis screen, hepatitis B surface antigen test, rubella IgG, urinalysis and culture, and varicella IgG (if status is unknown). The following tests are offered to all patients: hemoglobin electrophoresis, aneuploidy screening, cystic fibrosis carrier screening, spinal muscle atrophy carrier screening, maternal serum alpha fetoprotein (15-22 weeks). They also list the following among their recommendations (VA & DOD, 2023):

- "We recommend screening for use of tobacco and nicotine products, alcohol, cannabis, illicit drugs, and inappropriate use of prescription medication." [Strong]
- "We recommend screening for depression periodically using a standardized tool such as the Edinburgh Postnatal Depression Scale or the 9- item Patient Health Questionnaire periodically during pregnancy and postpartum." [Strong]
- "We recommend offering non-invasive prenatal testing as the prenatal screening test of choice for all patients with singleton pregnancies who choose aneuploidy screening." [Weak]
- "We suggest non-invasive prenatal testing for patients with twin pregnancies who choose aneuploidy screening." [Weak]
- "We recommend considering the use of fetal fibronectin testing as a part of the evaluation strategy in [individuals] between 24 and 34 6/7 weeks gestation with signs and symptoms of preterm labor, particularly in facilities where the result might affect management of delivery." [Strong]
- "We suggest patients who have undergone bariatric surgery be evaluated for nutritional deficiencies and need for nutritional supplementation where indicated (e.g., vitamin B12, folate, iron, calcium)." [Weak]

Health Resources & Services Administration (HRSA)

The HRSA recommends the following:

- "Screening pregnant individuals for gestational diabetes mellitus after 24 weeks of gestation (preferably between 24 and 28 weeks of gestation)
- Individuals with risk factors for diabetes mellitus be screened for preexisting diabetes before 24 weeks of gestation—ideally at the first prenatal visit.
- Screening for HIV is recommended for all pregnant [individuals] upon initiation of prenatal care with retesting during pregnancy based on risk factors.

- Rapid HIV testing is recommended for pregnant [individuals] who present in active labor with an undocumented HIV status" (HRSA, 2022).

Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, please visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

The FDA has approved many tests for conditions that can be included in a prenatal screening, such as HSV, chlamydia, gonorrhea, syphilis, and diabetes. Additionally, many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
80055	Obstetric panel This panel must include the following: Blood count, complete (CBC), automated and automated differential WBC count (85025 or 85027 and 85004) OR Blood count, complete (CBC), automated (85027) and appropriate manual differential WBC count (85007 or 85009) Hepatitis B surface antigen (HBsAg) (87340) Antibody, rubella (86762) Syphilis test, non-treponemal antibody; qualitative (eg, VDRL, RPR, ART) (86592) Antibody screen, RBC, each serum technique (86850) Blood typing, ABO (86900) AND Blood typing, Rh (D) (86901)
80081	Obstetric panel (includes HIV testing) This panel must include the following: Blood count, complete (CBC), and automated differential WBC count (85025 or 85027 and 85004) OR Blood count, complete (CBC), automated (85027) and appropriate manual differential WBC count (85007 or 85009) Hepatitis B surface antigen (HBsAg) (87340) HIV-1 antigen(s), with HIV-1 and HIV-2 antibodies, single result (87389) Antibody, rubella (86762) Syphilis test, non-

CPT	Code Description
	treponemal antibody; qualitative (eg, VDRL, RPR, ART) (86592) Antibody screen, RBC, each serum technique (86850) Blood typing, ABO (86900) AND Blood typing, Rh (D) (86901)
81001	Urinalysis, by dip stick or tablet reagent for bilirubin, glucose, hemoglobin, ketones, leukocytes, nitrite, pH, protein, specific gravity, urobilinogen, any number of these constituents; automated, with microscopy
81002	Urinalysis, by dip stick or tablet reagent for bilirubin, glucose, hemoglobin, ketones, leukocytes, nitrite, pH, protein, specific gravity, urobilinogen, any number of these constituents; non-automated, without microscopy
81003	Urinalysis, by dip stick or tablet reagent for bilirubin, glucose, hemoglobin, ketones, leukocytes, nitrite, pH, protein, specific gravity, urobilinogen, any number of these constituents; automated, without microscopy
81007	Urinalysis; bacteriuria screen, except by culture or dipstick
81015	Urinalysis; microscopic only
82677	Estriol
82731	Fetal fibronectin, cervicovaginal secretions, semi-quantitative
82947	Glucose; quantitative, blood (except reagent strip)
82950	Glucose; post glucose dose (includes glucose)
82951	Glucose; tolerance test (GTT), 3 specimens (includes glucose)
82962	Glucose, blood by glucose monitoring device(s) cleared by the FDA specifically for home use
83036	Hemoglobin; glycosylated (A1C)
84702	Gonadotropin, chorionic (hCG); quantitative
84703	Gonadotropin, chorionic (hCG); qualitative
84704	Gonadotropin, chorionic (hCG); free beta chain
85004	Blood count; automated differential WBC count
85007	Blood count; blood smear, microscopic examination with manual differential WBC count
85009	Blood count; manual differential WBC count, buffy coat
85014	Blood count; hematocrit (Hct)
85018	Blood count; hemoglobin (Hgb)
85025	Blood count; complete (CBC), automated (Hgb, Hct, RBC, WBC and platelet count) and automated differential WBC count
85027	Blood count; complete (CBC), automated (Hgb, Hct, RBC, WBC and platelet count)
85032	Blood count; manual cell count (erythrocyte, leukocyte, or platelet) each
85041	Blood count; red blood cell (RBC), automated

CPT	Code Description
86480	Tuberculosis test, cell mediated immunity antigen response measurement; gamma interferon
86580	Skin test; tuberculosis, intradermal
86592	Syphilis test, non-treponemal antibody; qualitative (eg, VDRL, RPR, ART)
86593	Syphilis test, non-treponemal antibody; quantitative
86631	Antibody; Chlamydia
86632	Antibody; Chlamydia, IgM
86704	Hepatitis B core antibody (HBcAb); total
86706	Hepatitis B surface antibody (HBsAb)
86762	Antibody; rubella
86780	Antibody; Treponema pallidum
86787	Antibody; varicella-zoster
86803	Hepatitis C antibody
86804	Hepatitis C antibody; confirmatory test (eg, immunoblot)
86850	Antibody screen, RBC, each serum technique
86900	Blood typing, serologic; ABO
86901	Blood typing, serologic; Rh (D)
87077	Culture, bacterial; aerobic isolate, additional methods required for definitive identification, each isolate
87081	Culture, presumptive, pathogenic organisms, screening only;
87086	Culture, bacterial; quantitative colony count, urine
87088	Culture, bacterial; with isolation and presumptive identification of each isolate, urine
87110	Culture, chlamydia, any source
87270	Infectious agent antigen detection by immunofluorescent technique; Chlamydia trachomatis
87320	Infectious agent antigen detection by immunoassay technique, (eg, enzyme immunoassay [EIA], enzyme-linked immunosorbent assay [ELISA], immunochemiluminometric assay [IMCA]) qualitative or semiquantitative, multiple-step method; Chlamydia trachomatis
87340	Infectious agent antigen detection by immunoassay technique, (eg, enzyme immunoassay [EIA], enzyme-linked immunosorbent assay [ELISA], immunochemiluminometric assay [IMCA]) qualitative or semiquantitative, multiple-step method; hepatitis B surface antigen (HBsAg)
87341	Infectious agent antigen detection by immunoassay technique, (eg, enzyme immunoassay [EIA], enzyme-linked immunosorbent assay [ELISA], immunochemiluminometric assay [IMCA]) qualitative or semiquantitative, multiple-step method; hepatitis B surface antigen (HBsAg) neutralization

CPT	Code Description
87389	Infectious agent antigen detection by immunoassay technique, (eg, enzyme immunoassay [EIA], enzyme-linked immunosorbent assay [ELISA], fluorescence immunoassay [FIA], immunochemiluminometric assay [IMCA]) qualitative or semiquantitative; HIV-1 antigen(s), with HIV-1 and HIV-2 antibodies, single result
87490	Infectious agent detection by nucleic acid (DNA or RNA); Chlamydia trachomatis, direct probe technique
87491	Infectious agent detection by nucleic acid (DNA or RNA); Chlamydia trachomatis, amplified probe technique
87590	Infectious agent detection by nucleic acid (DNA or RNA); Neisseria gonorrhoeae, direct probe technique
87591	Infectious agent detection by nucleic acid (DNA or RNA); Neisseria gonorrhoeae, amplified probe technique
87592	Infectious agent detection by nucleic acid (DNA or RNA); Neisseria gonorrhoeae, quantification
87653	Infectious agent detection by nucleic acid (DNA or RNA); Streptococcus, group B, amplified probe technique
87800	Infectious agent detection by nucleic acid (DNA or RNA), multiple organisms; direct probe(s) technique
87802	Infectious agent antigen detection by immunoassay with direct optical observation; Streptococcus, group B
87810	Infectious agent antigen detection by immunoassay with direct optical observation; Chlamydia trachomatis
87850	Infectious agent antigen detection by immunoassay with direct optical observation; Neisseria gonorrhoeae
G0306	Complete CBC, automated (HgB, HCT, RBC, WBC, without platelet count) and automated WBC differential count
G0307	Complete (CBC), automated (HgB, HCT, RBC, WBC; without platelet count)
G0472	Hepatitis C antibody screening, for individual at high risk and other covered indication(s)
S3652	Saliva test, hormone level; to assess preterm labor risk

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

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Revision History

Revision Date	Summary of Changes
09/04/2024	Reviewed and Updated: Updated background, guidelines, and evidence-based scientific references. Literature review necessitated the following changes in coverage criteria: Updated recommended testing type for HIV(CC1a), Hep B (CC1d), and Hep C(CC1f), updated spelling of N. gonorrhoeae (CC1c, CC2), CC1k edited for clarity and consistency. Now reads: "a) Antigen/antibody combination assay screening for HIV infection. c) Screening for Neisseria gonorrhoeae infection. d) Triple panel screening (HBsAg, anti-HBs, total anti-HBc) for hepatitis B. f) Antibody screening for hepatitis C. k) Screening for Group B streptococcal disease (once per pregnancy; recommended during gestational weeks 36 to 37)." CC2 edited for clarity and consistency. CC3 edited for clarity on coverage in relation to setting (Avalon only manages ambulatory settings). Now reads: "3) For individuals who are pregnant with singleton or twin pregnancies and who are presenting in the ambulatory setting with signs or symptoms of preterm labor, a fetal fibronectin (FFN) assay MEETS COVERAGE CRITERIA." Rewording of CC3 makes CC5 redundant, results in the removal of CC5: "5) For all other situations not described above, FFN assays DO NOT MEET COVERAGE CRITERIA." Added CPT code 87389 Removed CPT code 83020, 83021, 85048, 86701, 86702, 86703, G0432, G0433, G0435; 0167U (deleted code; effective date 10/1/2024)

Prescription Medication and Illicit Drug Testing in the Outpatient Setting

Policy Number: AHS – T2015 – Prescription Medication and Illicit Drug Testing in the Outpatient Setting	Prior Policy Name and Number, as applicable: • AHS-T2015- Opioids Testing in Pain Management and Substance Abuse • AHS-T2015-Toxicology
Initial Presentation Date: 06/06/2015 Revision Date: 03/06/2024	

Policy Description

Abuse of both prescription and illicit drugs is extremely common. Drugs of abuse (DOA) may be defined as "a drug, chemical, or plant product that is known to be misused for recreational purposes," which can include drugs such as pain relievers that have legitimate prescriptions. Drug tests may be performed for a variety of reasons, such as compliance with treatment program or medical regimen. Numerous biological substances, such as blood, hair, or saliva may be tested, but urine is the most commonly tested biological substance in drug tests (Hoffman, 2023).

This policy addresses clinical toxicology in the outpatient setting and does not address forensic testing or therapeutic drug monitoring (TDM). Forensic drug testing is used for legal proceedings and requires secondary confirmatory testing (Jones, 2016). TDM "involves sampling of plasma or serum drug levels to determine optimal drug dosing" (Eaton & Lyman, 2022).

Related Policies

Policy Number	Policy Title
	Not applicable

Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

This policy concerns only coverage criteria and does not describe or define the legal responsibility of providers. Providers should refer to state and federal laws for such guidance.

This policy does not address the use of drug testing in the following circumstances:

A. State, federally regulated, and legally mandated drug testing (i.e., court-ordered drug screening, forensic examinations).

B. Non-forensic testing for commercial driver's licensing or any other job-related testing (i.e., as a prerequisite for employment or as a means for continuation of employment).

C. As a component of routine physical/medical examination.

D. As a component of care rendered in an urgent/emergency situation.

E. As a routine component of a behavioral health assessment.

PRESUMPTIVE DRUG SCREENING USING URINE SAMPLES

1) Presumptive drug screening using urine samples (qualitative, semi-quantitative or quantitative) **MEETS COVERAGE CRITERIA** in **any** of the following situations:
    a) To assess an individual being treated for chronic, non-cancer pain when clinical evaluation of the individual (history/signs/symptoms) suggests the use of non-prescribed medications or illegal substances:
        i) Prior to initiating chronic opioid pain therapy in chronic non-cancer pain to determine if the individual has been exposed to controlled substances or potentially confounding illicit drugs.
        ii) To verify an individual's compliance with treatment or identify undisclosed drug abuse as part of routine monitoring for individuals who are receiving treatment for non-cancer chronic pain with prescription opioid pain medication. The random testing interval and drugs selected for testing should be based on the individual's history, condition, and treatment, as documented in the medical record.
            (a) Monitoring of low risk (as defined by a risk assessment tool) individuals on chronic opioid therapy, up to one (1) time per year after initiation of therapy.
            (b) Monitoring of moderate risk (as defined by a risk assessment tool) individuals on chronic opioid therapy, up to two (2) times per year after initiation of therapy.
            (c) Monitoring of high risk (as defined by a risk assessment tool) individuals on chronic opioid therapy, up to four (4) times per year after initiation of therapy.

(d) For individuals with aberrant behavior (lost prescriptions, multiple requests for early refills, and opioids from multiple providers, unauthorized dose escalation, apparent intoxication, etc.), testing at the time of visit meets coverage criteria.

b) In pregnant individuals at high-risk for substance abuse in whom the suspicion of drug use exists based on the answers to substance abuse screening questions or as indicated by information from the prescription drug monitoring program (PDMP), as documented in the medical record.

c) In newborns when there is a history of maternal substance abuse or agitated/altered mental status in the birthing parent.

d) In candidates for organ transplant who have a history of substance abuse (to demonstrate abstinence prior to transplant).

e) In individuals with a suspicion of or a diagnosis of mental illness (e.g., anxiety disorders, schizophrenia, major depressive disorder, mood disorders, suicidal ideations, substance abuse disorder).

f) In individuals with attention-deficit hyperactivity and disruptive behavior disorders.

g) In cancer patients on opioid pain medication.

h) In individuals with epilepsy.

i) For the management and compliance monitoring of an individual under treatment for substance abuse or dependence at the following frequency (after baseline at initial evaluation) and must be documented in the patient's medical record:

   i) For patients with 0 to 90 consecutive days of abstinence, random qualitative drug testing at a frequency of 1 to 2 per week.

   ii) For patients with > 90 consecutive days of abstinence, random qualitative drug testing at a frequency of 1 to 3 per month.

j) In individuals where substance abuse is in the differential diagnosis of the presenting conditions.

<u>DEFINITIVE DRUG TESTING</u>

2) Confirmatory/definitive qualitative or quantitative drug testing (up to seven drug classes) **MEETS COVERAGE CRITERIA** when laboratory-based definitive drug testing is specifically requested, the rationale is documented by the patient's treating physician, and any of the following conditions are met:

   a) The result of the presumptive drug screen is different than that suggested by the patient's medical history, their clinical presentation, or patient's own statement (e.g., test was negative for prescribed medications, test was positive for prescription drug with abuse potential which was not prescribed, test was positive for an illegal drug).

   b) For diagnosing and monitoring individuals with substance use disorder or dependence, when accurate and reliable results are necessary for treatment decisions:

      i) Individuals with 0 to 30 consecutive days of abstinence, random definitive drug testing at a frequency not to exceed 1 per week.

ii) Individuals with 31 to 90 consecutive days of abstinence, random definitive drug testing at a frequency of 1 to 3 per month. No more than 3 definitive drug tests in one month will be allowed.

iii) Individuals with greater than 90 consecutive days of abstinence, definitive drug testing at a frequency of 1 to 3 every three months. No more than 3 definitive drug tests in a 3-month period will be allowed.

c) For monitoring of individuals on opioid therapy (to ensure adherence to the therapeutic plan, for treatment planning, and for detection of other, non-prescribed opioids).

d) A presumptive test does not exist or does not adequately detect the specific drug or metabolite to be tested (e.g., specific drugs within the amphetamine, barbiturate, benzodiazepine, tricyclic antidepressants, and opiate/opioid drug classes, as well as synthetic/analog or "designer" drugs).

e) To definitively identify specific drugs in a large family of drugs.

f) To identify drugs when a definitive concentration of a drug is needed to guide management.

3) When laboratory-based definitive drug testing is requested for larger than seven drug classes panels, confirmatory/definitive qualitative or quantitative drug testing **DOES NOT MEET COVERAGE CRITERIA**.

4) Confirmatory/definitive qualitative or quantitative or presumptive (qualitative, semi-quantitative or quantitative) drug testing using proprietary tests (e.g., CareView360) **DOES NOT MEET COVERAGE CRITERIA**.

SPECIMEN VALIDITY TESTING

5) Specimen validity testing (e.g., urine specific gravity, urine creatinine, pH, urine oxidant level, genetic identity testing [e.g., NextGen Precision™ Testing]) **DOES NOT MEET COVERAGE CRITERIA**.

NOTES:

Documentation Requirements

The patient's medical record must contain documentation that fully supports the medical necessity for drug testing. This documentation includes, but is not limited to, relevant medical history, physical examination, and results of pertinent diagnostic tests or procedures.

Reimbursement

1. The following **IS** reimbursed (see complete Coverage Criteria in Letters A and B, Section III above) for:
   a. Presumptive drug screening based upon appropriate clinical criteria (qualitative, semi-quantitative or quantitative);
   b. Definitive drug testing (qualitative or quantitative) for up to seven drug classes when the presumptive drug screening meets one of the following criteria:
      i. The test was negative for prescribed medications, or
      ii. Positive for a prescription drug with abuse potential which was not prescribed, or
      iii. Positive for an illegal drug, or

iv. A presumptive test does not exist or does not adequately detect the specific drug or metabolite to be tested

c. Blood specimens in patients with anuric Chronic Renal Failure.

1. The following **IS NOT REIMBURSED**:

   a. Any AMA definitive drug class codes
   b. Same-day testing of the same drug or metabolites from two different samples (e.g. both a blood and a urine specimen) by either presumptive or definitive analyses
   c. Blanket orders or routine standing orders for all patients in the physician's practice

2. Only urine or oral fluid specimens will be covered except blood specimen will be covered for patients with anuric Chronic Renal Failure.

3. Confirmatory/definitive testing should be supported by documentation of rationale in the patient's medical record.

4. More than one presumptive test result per patient per date of service regardless of the number of billing providers **IS NOT REIMBURSED**:

   a. It is not reasonable or necessary for a provider to perform qualitative point-of-care testing and also order presumptive testing from a reference laboratory on the same specimen.
   b. It is not reasonable or necessary for a provider to perform presumptive immunoassay testing and also order presumptive immunoassay testing from a reference laboratory with or without reflex testing on the same specimen.

Table of Terminology

Term	Definition
6-AM	6-acetylmorphine
6-MAM	6-monoacetylmorphine
AACAP	American Academy of Child and Adolescent Psychiatry
AACC	American Association for Clinical Chemistry
AAFP	American Academy of Family Physicians
AAN	American Academy of Neurology
AAPM	American Academy of Pain Medicine
AATOD	American Association for The Treatment of Opioid Dependence Inc.
ACOEM	American College of Occupational and Environmental Medicine
ACOG	American College of Obstetricians and Gynecologists
ADAC	Anxiety Disorders Association of Canada
ADHD	Attention-deficit/hyperactivity disorder
AMA	American Medical Association
AMDG	Agency Medical Directors' Group
APA	American Psychiatric Association
ASAM	American Society of Addiction Medicine
ASIPP	American Society of Interventional Pain Physicians
AUDIT-C	Alcohol use disorders identification test-consumption
BD	Bipolar disorder
CDC	Centers for Disease Control and Prevention

CLIA ’88	Clinical Laboratory Improvement Amendments Of 1988
CMS	Centers For Medicare and Medicaid Services
COT	Chronic opioid treatment
CPS	Canadian Paediatric Society
CYP2D6	Cytochrome P450 2D6
DNA	Deoxyribonucleic acid
DOA	Drugs of abuse
DOD	Department Of Defense
DVA	Department of Veterans Affairs
EDDP	2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine
EIAs	Enzyme immunoassays
EMIT	Enzyme multiplied immunoassay technology
FDA	Food and Drug Administration
FSMB	Federation Of State Medical Boards
GABA	Gamma aminobutyric acid
GAD	Generalized anxiety disorder
GC	Gas chromatography
GHB	Gamma-hydroxybutyrate
HHS	Department Of Health and Human Services
HIV	Human immunodeficiency virus
JA	Joint arthroplasty
LC	Liquid chromatography
LCD	Local coverage determinations
LDTs	Laboratory-developed tests
LSD	Lysergic acid diethylamide
MDMA	3,4-methylenedioxymethamphetamine
MS	Mass spectrometry
MTF	Monitoring the future
NACB	National Academy of Clinical Biochemistry
NICE	National Institute for Health and Care Excellence
NIDA	National Institute of Drug Abuse
NMDA	N-methyl-d-aspartic acid
NOUGG	National Opioid Use Guideline Group
NSDUH	National Survey on Drug Use and Health
OASAS	Office of Addiction Services and Supports
OTPs	Opioid treatment programs
OUD	Opioid use disorder
PCP	Phencyclidine
PDMP	Prescription drug monitoring program
POC	Point-of-care
SAD	Social anxiety disorder
SAMHSA	Substance Abuse and Mental Health Services Administration
SASQ	Single item alcohol screening questionnaire
SOAPP	Screener and opioid assessment for patients with pain
SOGC	Society Of Obstetricians and Gynaecologists of Canada
SUD	Substance use disorder

TCAs	Tricyclic antidepressants
TDM	Therapeutic drug monitoring
THC	Tetrahydrocannabinol
TLC	Thin layer chromatography
UDM	Urine drug monitoring
UDS	Urine drug screening
UDT	Urine drug testing
UMHS	University of Michigan Health System
VA/DOD	Department Of Veterans Affairs/Department of Defense
WFSBP	World Federation of Societies of Biological Psychiatry
WHO	World Health Organization

Scientific Background

According to the National Center for Drug Abuse Statistics, as many as 31.9 million Americans 12 or older used an illicit drug in the last 30 days, which corresponds to 11.7% of Americans overall and 39% for young adults from 18 to 25. 10.1 million misused opioids in the previous year, with 9.7 million misusing prescription pain relievers. Approximately 9.5 million adults had a concurrent mental illness and substance abuse disorder in the previous year (National Center for Drug Abuse Statistics, 2022) . A drug of abuse (DOA) may be defined as "a drug, chemical, or plant product that is known to be misused for recreational purposes," which can include drugs, such as pain relievers, that have legitimate prescriptions. Drug testing may be performed for several reasons. For example, patients in areas including pain management, substance abuse treatment, and psychiatric treatment have a higher propensity for substance abuse and must be monitored as such (Hoffman, 2023).

DOA screening varies in composition between countries. In the U.S., typical DOA screening tests encompass amphetamine, cocaine, marijuana/tetrahydrocannabinol (THC), opioids, and phencyclidine (PCP) as included in the United States' Drug-Free Workplace Act of 1988; these DOA are often referred as the SAMHSA 5, named after the Substance Abuse and Mental Health Services Administration (Hoffman, 2023; Phan et al., 2012). Although the drug trends have changed dramatically since 1988, these five have remained on the basic drug screen used across the U.S. The U.S. Department of Defense (DOD) removed PCP from its routine screening but added benzodiazepines, amphetamine derivatives, common barbiturates, synthetic and semisynthetic opioids, lysergic acid diethylamide (LSD), and synthetic cannabinoids. Other countries or regions, such as Australia and the European Union, also include testing for benzodiazepines and wider range of opioids (Hoffman, 2023). The American Society of Addiction Medicine (ASAM) recommends drug-testing panels based on "the patient's drug of choice, prescribed medications, and drugs commonly used in the patient's geographic location and peer group" rather than relying on the SAMHSA 5 (ASAM, 2017).

The testing performed could be qualitative, semi-quantitative or quantitative, presumptive, or definitive. Qualitative refers to testing for the presence of a given analyte, semi-quantitative reports if the analyte is present above or below a certain threshold, and quantitative reports the exact amount of an analyte. Presumptive drug testing is used to identify use or non-use of a drug or a drug class, but this type of testing cannot distinguish between structural isomers. Definitive drug testing usually refers to a more definitive methodology, such as mass spectrometry or chromatography, because these methods can identify use or non-use of a specific drug and/or its associated metabolites. Both types of drug testing can be either quantitative or qualitative (Jannetto & Langman, 2018). The frequency of testing is usually

determined by the providers; testing may be random or scheduled depending on the provider's objectives (Becker & Starrels, 2023).

Urine drug tests are the most common method of drug testing for several reasons. Unlike blood or saliva, the window of detection of most drugs is longer in urine; moreover, urine tests are inexpensive, noninvasive, and convenient to use while still maintaining acceptable statistical validity. Salivary testing can provide a higher rate of false-negative results, especially for individuals who smoke. Urine may provide more objective assessment of drug levels compared to purely clinician evaluation or a patient self-report (Becker & Starrels, 2023). A disadvantage of urine testing is "a high risk of adulteration of the sample by the patient to avoid detection of non-compliance with the therapeutic regimen" (AACC, 2017). The table below, adapted from Hoffman (2023), summarizes urine drug testing assays for several drugs.

Drug	Time frame for testing	Substance detected	Potential False-Positives (Varies by Assay)
Amphetamine	1-2 days (acute exposure) 2-4 days (chronic exposure)	Amphetamine	Poor specificity due to structural similarities to many drugs, herbal supplements, and medications, including many nasal decongestants.
Benzodiazepines (Note: No single assay is known to detect all benzodiazepines.)	1-5 days for most benzodiazepines 2-30 days for diazepam	Oxazepam (most common) Various metabolites	Oxaprozin
Cocaine	2 days (acute exposure) 7 days (chronic exposure)	Benzoylecgonine	Coca tea, coca leaves
GHB	< 24 hours	GHB	"Endogenous neurotransmitter naturally present in minute quantities"
Ketamine	1-3 days	Ketamine, norketamine	
LSD	1-3 days	2-oxo-3-hydroxy-LSD	
Marijuana (Note: Synthetic cannabinoids are not usually detected by routine urine assays.)	1-3 days (acute exposure) >1 month (chronic exposure)	11-nor-9-carboxy-Δ^9-THC	Hemp-containing foods or hemp products (e.g. hemp soap) in rare cases
Opioids (Synthetic opioids are not	1-3 days	Morphine and all natural opioids (e.g. codeine)	Poppy seeds (Note: The threshold for urine detection has been substantially

detected by routine opioid screening, though specific assays such as buprenorphine are available.)			raised to decrease the likelihood of poppy seed false-positives.)
Methadone	1-5 days	Methadone EDDP	Doxylamine
PCP	4-7 days	PCP	Dextromethorphan, diphenhydramine, doxylamine, ketamine, tramadol, venlafaxine

Presumptive urine drug testing (UDT) typically uses an immunoassay where antibodies detect the drug or drug metabolite. This testing can be either qualitative, showing only a positive or negative finding, or semi-quantitative. Immunoassays offer fast turnaround times but can also give false-positive or false-negative results. Federal Workplace Drug Testing Programs usually use higher cutoff values to avoid false-positive results, but this can increase the likelihood of false-negatives (AACC, 2017). One study reports a false-negative rate of 28% for detecting benzodiazepines (Manchikanti et al., 2010). Another approach is to utilize orthogonal testing where an initial immunoassay is followed by a spectroscopic assay. This can be used for monitoring compliance in pain management therapy (AACC, 2017). Regardless, proper interpretation of results is imperative. Inadequate physician knowledge of interpretation can limit the efficient use of UDT (Pesce et al., 2012); in fact, a single study found that 25 of 88 (28%) of UDT results were susceptible to provider interpretation error when compared to the laboratory toxicologist's interpretation (Chua et al., 2020).

Presumptive point-of-care (POC) testing is also available. POC tests use either a urine or saliva sample to screen for drugs in an immunoassay. Like laboratory-based immunoassays, POC testing has lower sensitivity and specificity than definitive drug tests; however, they can provide immediate results to the physician where a negative result typically rules out DOA and a positive result requires confirmatory testing (AACC, 2017). False-positive and false-negative results are even more problematic in POC testing than laboratory-based immunoassays. The clinician must be cognizant of medications—both prescribed and over-the-counter—that can trigger false-positives; for example, over-the-counter nasal inhalers can contain active ingredients that give a potential false-positive for methamphetamine. Moreover, POC testing may not be capable of detecting medications that are metabolites of parent medications (Pesce et al., 2012).

Definitive drug testing typically uses chromatographic and spectroscopic methodologies, including gas chromatography (GC) or liquid chromatography (LC) and tandem mass spectrometry (MS). According to the American Association for Clinical Chemistry (AACC), MS-based assays are traditionally considered the gold standard even though they are both more labor- and time-intensive. Whereas immunoassay-based assays usually only detect a class of compounds, MS-based assays can detect specific drugs in urine samples (AACC, 2017).

Opioids

Opioids are the standard of care for moderate to severe pain, and primarily work by stimulating the μ, δ, or κ opioid receptors in the central nervous system and throughout the body (Owusu Obeng et al., 2017). The stimulation of these receptors typically causes blocking of pain neurotransmitters such as glutamate and blocks the release of GABA, thereby producing extra dopamine. This extra dopamine also creates a pleasurable effect and possible euphoria (Trescot et al., 2008).

However, due to their mechanism of action, opioids and other pain relievers can cause addiction and are widely abused. According to the CDC, over 142 million prescriptions for opioids were written in 2020 (CDC, 2022b). Although the overall trend in annual opioid prescribing rates have been falling from the peak in 2012 of 81.3 prescriptions per 100 persons to 43.3 per 100 in the most recently reported year (2020)(CDC, 2022b), opioid abuse is still extremely widespread and considered an "epidemic" in the United States. According to the CDC, in 2019, a 4% increase in the number of age-adjusted rate of drug overdose deaths occurred, and 70.6% of all drug overdose deaths involved the use of opioids (CDC, 2022a). In 2019, a total of 70,630 drug overdose deaths occurred in the United States (CDC, 2022a). The CDC monitors the number of deaths and nonfatal overdoses of opioids in four categories (CDC, 2017):

- Natural/semi-synthetic opioids, such as morphine and oxycodone, respectively
- Methadone
- Synthetic opioids other than methadone
- Heroin

Immunoassay-based screening tests for opioids typically detect morphine, a common metabolite in natural opioids and heroin; however, synthetic opioids, such as fentanyl, methadone, and tramadol, and semisynthetic opioids, including hydrocodone and oxycodone, are not detected using routine opioid screening. These drugs are detected using a specific screening assay. Previously, poppy seed consumption triggered false-positive results, so the U.S. Substance Abuse Mental Health Services Administration (SAMHSA) raised the urine threshold for morphine from 300 ng/mL to 2000 ng/mL. Additionally, heroin can be distinguished from poppy seed exposure by testing for 6-monoacetylmorphine (6-MAM) (Hoffman, 2023). 6-MAM has a short half-life before it metabolizes to morphine; the absence of 6-MAM does not rule heroin use (Pesce et al., 2018).

Non-Opioid Medications Used in Chronic Pain Management

Other non-opioid medications can be used in chronic pain management, including antidepressants, anticonvulsants, neuroleptics, antispasmodics, and muscle relaxants. Tricyclic antidepressants (TCAs), such as nortriptyline, are used in pain management even though the analgesic mechanism is unknown. At times, TCAs may be used as adjuncts to opioid therapy to potentiate the analgesic effect of the opioid for individuals suffering from severe pain and/or diabetic neuropathy. Certain newer anticonvulsants, such as pregabalin and gabapentin, can be used as first-line agents in chronic pain treatment due to favorable side effect profiles. Neuroleptics can be used, especially for patients with psychotic symptomology, but these drugs can have undesirable long-term side effects, including akathisia and tardive dyskinesia. Pain due to muscle spasms in certain individuals may be relieved using muscle relaxants and antispasmodics, including baclofen. These non-opioid medications may be monitored for compliance similarly to their opioid counterparts in patients. The table below lists examples of common non-opioid medications that may be used for pain management (AACC, 2017).

Antidepressants	**Anticonvulsants**	**Neuroleptics**	**Antispasmodics & Muscle Relaxants**
Doxepin	Phenytoin	Fluphenazine	Baclofen

Amitriptyline Imipramine Nortriptyline Desipramine Venlafaxine Duloxetine	Gabapentin Pregabalin Carbamazepine Oxcarbazepine Clonazepam	Haloperidol Chlorpromazine Perphenazine	Cyclobenzaprine Carisoprodol

Benzodiazepines and Barbiturates

Due to their anxiolytic and hypnotic properties, tranquilizers, such as benzodiazepines—including Xanax, Valium, and Restoril—have an especially high rate of abuse as they are frequently prescribed for common disorders, such as anxiety and insomnia. Benzodiazepine intoxication has similar features to alcohol intoxication; severe overdose leads to respiratory depression and eventual anoxic brain damage or death (Weaver, 2015). Benzodiazepines consist of approximately 90% of tranquilizer abuse (Becker & Starrels, 2023) and consisted of approximately 30% of deaths from a pharmaceutical agent in 2010 (Jones et al., 2013). Benzodiazepines are not typically included in the standard urine screening for DOA, but the most common test for benzodiazepines identifies metabolites of 1,4-benzodiazepines like oxazepam. Benzodiazepines that do not metabolize in this manner (such as Xanax) may not be detected. Furthermore, a positive test only indicates a recent exposure to the drug indicated (Greller & Gupta, 2024). The HIV treatment efavirenz gives a false-positive result in benzodiazepine screening; in fact, one study reported that 98% of urine samples of individuals on efavirenz gave a false-positive as compared to only 2% of the control group (Blank et al., 2009). Testing for benzodiazepines is particularly important if opioids or alcohol are involved; 28% of all prescription opioid overdoses in 2015 involved benzodiazepines (Kandel et al., 2017). And, false negative results are often seen in a pain management population in patients prescribed lorazepam and clonazepam because benzodiazepine immunoassays are inadequately sensitive (Jannetto et al., 2017).

Although barbiturates, another class of sedatives, are not prescribed as much as in the past, they are still an abusable drug and have use as an anesthetic and anticonvulsant. Barbiturates are also frequently prescribed for headaches, which can lead to physical withdrawal in the form of recurrent headaches (Weaver, 2015). Similar to benzodiazepines, barbiturates can produce a hypnotic and relaxing effect, but euphoria may be a side effect depending on dose (Eskridge & Guthrie, 1997). Its harmful side effects are similar to those of benzodiazepine poisoning (e.g. respiratory depression, slowed mental state) (Greller & Gupta, 2024). The barbiturate immunoassay typically detects secobarbital; the most frequently prescribed barbiturates of phenobarbital, primidone, and butalbital are detected well by barbiturate immunoassays (Algren & Christian, 2015). POC tests, such as the Instant-View® Barbiturate Urine Test, can be used for initial screening but should have confirmatory testing for positive results. According to its package insert, besides phenobarbital, "this test is designed to detect unchanged secobarbital in the urine; however, as with some other analytical methods such as EMIT and RIA, this assay can also detect other commonly encountered barbiturates, depending on the concentration of drug present in the sample. With standard single dose of secobarbital, pentobarbital, or amobarbital, positive results may be identified from 30 hours to 76 hours (ALFA, 2019)." A positive response rate of detection is reported with minimal concentrations of 200 – 300 ng/mL, depending on the barbiturate. The Wondfo Barbiturates Urine Test is another FDA approved POC test which provides results in five minutes. This test can identify 16 drugs including barbiturates and benzodiazepines with a single testing strip (Wondfo, 2020).

Amphetamines

Stimulants, including amphetamines and drugs prescribed for attention-deficit/hyperactivity disorder (ADHD), can be abused due to their euphoric side effects (Moeller et al., 2017). Although there are many different kinds of stimulants, their primary mechanism of action is blocking the dopamine receptor or stimulating release of dopamine (Kampman, 2023). Amphetamine side effects include tachycardia, high blood pressure, and agitation; severe overdose may lead to seizures, hallucinations, or paranoia (Becker & Starrels, 2023). UDTs for amphetamines, such as the DRI® Amphetamines Assay, are immunoassays that detect amphetamine and/or methamphetamine. The DRI® Amphetamines Assay has cutoff levels of 500 ng/mL for amphetamine and 1000 ng/mL for methamphetamine with 58.0% concordance between the immunoassay and GC/MS at the 500 ng/mL cutoff. The manufacturer states, "a positive result by this assay should be confirmed by another nonimmunological method such as GC, TLC or GC/MS (Microgenics, 2016)." Many false-positives can occur due to the high number of cross-reactants, including over-the-counter medicines and dietary supplements (Hoffman, 2023; Moeller et al., 2017). Even metformin, a medication prescribed to treat diabetes, can give false-positives although the mechanism of cross-reactivity is unknown (Fucci, 2012).

Phencyclidine

Phencyclidine (PCP), a *N*-methyl-*D*-aspartic acid (NMDA) receptor antagonist, is a dissociative anesthetic that can be abused for its euphoric properties. Also known as angel dust, PCP was the first non-natural man-made DOA (Bertron et al., 2018). Throughout the 1980s and 1990s, the use of PCP declined considerably; however, the Drug Abuse Warning Network has reported a 400% increase in emergency room visits due to PCP use in 2005 – 2011 (Moeller et al., 2017). PCP is typically screened using an immunoassay, and qualitative screening tests, such as CEDIA®, report a 100% reactivity at a PCP concentration of 25 ng/mL (Microgenics, 2015). Unfortunately, many compounds can interfere with the PCP immunoassay, including tramadol (Ly et al., 2012), dextromethorphan, alprazolam, clonazepam, and carvedilol (Rengarajan & Mullins, 2013). Some have reported that diphenhydramine (Benadryl®) also yields false-positive results (Brahm et al., 2010; Levine & Smith, 1990), but other studies have reported it to be statistically insignificant (Rengarajan & Mullins, 2013). The FDA approved Wondfo Phencyclidine Urine Test is an immunochromatographic assay which can identify PCP in human urine with a cutoff of 25 ng/mL (FDA, 2019). Nonetheless, this is considered a preliminary testing method and results should be confirmed with gas chromatography/mass spectrometry (GC/MS) techniques.

Marijuana/THC/Cannabinoids

According to the CDC, the most recent National Survey on Drug Use and Health (NSDUH), conducted by SAMHSA in 2013, showed that approximately 7.5% of people 12 years and older in the U.S. were current users of marijuana, which was up from 5.8% from 2007 (CDC, 2015). Moreover, the CDC reports that the Monitoring the Future (MTF) survey of 8th, 10th, and 12th graders in the U.S. shows that the rate of marijuana usage has remained steady for more than two decades even though many states and municipalities have changed their legislation. Approximately 5.8% of 12th graders reported daily use of marijuana (CDC, 2018a).

Immunoassays for marijuana do not detect tetrahydrocannabinol (THC) directly because THC rapidly metabolizes in vivo (within hours of use). Instead, these assays detect delta-9-THC, a metabolite, which can remain in either the serum or urine for days to weeks, depending on the extent of exposure (Hoffman, 2023). Older urine immunoassays for marijuana were prone to false-positive results (Altunkaya & Smith, 1990; Rollins et al., 1990), but current testing methods are less prone to false-positives (Hoffman, 2023). Due to the legalization of marijuana in certain locales as well as an increase in the potency of the THC in some strains of marijuana, fear of false-positive results due to second-hand

smoke has increased. Recent studies show, though, that this is unlikely. None of the individuals tested positive using an immunoassay with a cutoff level of more than 20 ng/mL provided that the room was well-ventilated. If the room was not ventilated, then four of six individuals tested positive after one hour of exposure if the immunoassay had a cutoff level of 20 ng/mL but only one individual tested positive at the federal cutoff level of 50 ng/mL under the same conditions (Cone et al., 2015; Herrmann et al., 2015; Moeller et al., 2017). False positive results for THC have also been caused by medications such as Pantoprazole (Vohra et al., 2019). However, Vohra et al. (2019) completed a small study (n=12) and found that oral proton pump inhibitors (such as Pantoprazole) did not cause false-positive THC results with the THC One Step Marijuana Test Strip. This test strip is a rapid chromatographic immunoassay which detects the delta-9-THC metabolite at a cutoff concentration of 50 ng/mL (Xlar, 2002).

Cocaine

Cocaine is an alkaloid produced biosynthetically by *Erthroxylum coca*, which is a plant native to western South America; for thousands of years, South Americans have chewed on the dried coca leaves or consumed coca tea to release cocaine in saliva (Drake & Scott, 2018). Pure cocaine was first isolated in the 1880s and was legal in the United States during the second half of the 19th century (Nelson & Odujebe, 2023). It was once a main ingredient of Coca-Cola. Cocaine is now illegal in the United States; importing coca leaves or coca tea is also illegal in the United States but is legal in other countries. Medicinal use of cocaine is typically limited to use in minor otolaryngologic procedures or as a topical anesthetic (Hoffman, 2023). It has vasoconstrictive properties, making it useful in limiting bleeding during nose and throat surgeries (Nelson & Odujebe, 2023).

Cocaine is a powerful nervous system stimulant and is highly addictive. According to the CDC (2019), cocaine was involved in almost one in every five overdose-related deaths in the United States in 2017, leading to 14,000 cocaine-related deaths. In 2016, almost five million Americans reported regular cocaine use, which was approximately 2% of the population (CDC, 2019).

Cocaine has three main metabolites--benzoylecgonine (>50 %), ecgonine methyl ester (32-49%) and norcocaine (5%) (Nelson & Odujebe, 2023). With benzoylecgonine identified as the major urinary metabolite of cocaine, it is usually tested for in blood, urine, hair, saliva, and meconium. Immunoassays are the most specific technique to detect the cocaine metabolite benzoylecgonine; false-positive results are very uncommon (Hoffman, 2023). Cocaine is metabolized very rapidly and may only be detectable in blood and urine for a few hours; however, benzoylecgonine can be detected in the urine for several days if cocaine use is intermittent or very heavy (Nelson & Odujebe, 2023). Appropriate urine tests distinguish between cocaine use and coca leave/tea use because different metabolites are formed from each. The DRI Cocaine Metabolite Assay, developed by Thermo Fisher, is an FDA-approved enzyme immunoassay that uses a specific antibody to detect benzoylecgonine in urine (FDA, 2018). This immunoassay has a concentration cutoff of 150 ng/mL-300 ng/mL.

Clinical Utility and Validity

For acute clinical management of most patients, DOA monitoring is of limited value. Studies have indicated that in specific settings DOA screening does have value, particularly for drug treatment programs, pain management, and/or psychiatric treatment. A large retrospective study (n = 470 patients) by Michna et al. (2007) showed that 20% of individuals in pain management programs tested positive for illicit substances when random screenings were performed. Further, Knezevic et al. (2017) performed a study showing the effect of urine drug testing on patient compliance. Five hundred patients provided supervised urine toxicology samples, 386 of which were compliant with prescribed

medications. The patients were educated about their results, and 77 of the noncompliant patients were tested again. Of these 77 patients, 49 had improved compliance (Knezevic et al., 2017). This supports the previous findings of a smaller study by Jamison and colleagues that reported a significant increase in compliance for high-risk chronic pain patients on opioid therapy when monitored by UDT (Jamison et al., 2010). Another study also supports UDT for patients on long-term opioid therapy by showing that "monitoring both urine toxicology and aberrant behavior in chronic-pain patients treated with opioids identified more problem patients than by monitoring either alone (Katz et al., 2003)."

These findings are considerably more favorable than those of the systematic review conducted by Starrels et al. (2010) of eleven different studies that found substantial variation in reduction of opioid misuse in patients with chronic pain. These researchers discovered that "the proportion of patients with opioid misuse after treatment agreements, urine drug testing, or both varied widely (3% to 43%)" and concluded that "relatively weak evidence supports the effectiveness of opioid treatment agreements and urine drug testing in reducing opioid misuse by patients with chronic pain (Starrels et al., 2010)." Even with the controversy, Christo et al. (2011) recommends using an algorithmic approach for urine drug testing where UDT is used to establish "a baseline measure of risk, as well as monitoring for compliance" (Christo et al., 2011), an approach also supported by the Texas Pain Society (Owen et al., 2012).

Additionally, other scenarios may utilize DOA testing to alter medical management. Patients with seizure disorders, such as epilepsy, who are on antiepileptic medications that block sodium channels (including phenytoin, lamotrigine, and carbamazepine) could benefit from DOA testing since cocaine can interact pharmacokinetically with these drugs (Smith & McBride, 1999; Wilfong, 2023). DOA screening to check for cocaine can be used prior to administration of beta-adrenergic antagonists. For patients who exhibit acute psychosis with no apparent or known cause, DOA screening can be used to detect possible stimulants (Hoffman, 2023; McClellan & Stock, 2013). Alternatively, psychiatric pre-administration acetaminophen or salicylate screening is deemed unnecessary by Farkas et al. (2021) following their multicenter retrospective study. The authors analyzed 33,439 tests over 10 years from three different Veteran's Administration emergency departments. There were no toxicity diagnoses. The authors suggest that the testing is "unnecessary and wasteful" (Farkas et al., 2021).

For monitoring a drug therapy regimen, some have proposed using quantitative, definitive testing (Couto et al., 2009, 2011; Kell, 1994; Pesce et al., 2012). Small studies by Couto and colleagues reported concordance correlation coefficients of 0.677 (n = 20) for assessing adherence to a hydrocodone regimen and 0.689 (n = 36) for an oxycontin regimen using normalized algorithms (Couto et al., 2009, 2011). Other studies have shown that due to the variability in pharmacokinetics, pharmacodynamics, and pharmacogenetics between individuals, such quantitative testing does not correlate to "patient compliance with a drug dosage using commercial algorithms" (Nafziger & Bertino, 2009). Another study by McEvoy et al. (2014) aiming to assess urine levels of aripiprazole and its metabolites for patients on an aripiprazole regimen, at best, only found an R^2 value of 0.7 even when adjusted for age, weight, sex, urine creatinine values, height, urine specific gravity, and dosage range. "Unadjusted urine levels of aripiprazole and metabolites are not strongly related to aripiprazole dosing…variance in urine metabolite levels accounted for by medication dose was relatively low for each individual drug/metabolite, [R^2] only 0.13 to 0.23 (McEvoy et al., 2014)." Even the study by Couto notes the limitations concerning pharmacogenetics, excluding any patient who was "determined to be poor, rapid, or ultra-rapid CYP2D6 metabolizers" (Couto et al., 2011).

A study performed by Snyder et al. (2017) assessed the accuracy of enzyme immunoassays (EIAs) for patients being treated for chronic pain. A total of 530 patient samples were taken, and the immunoassays were evaluated for accuracy. The EIAs showed an overall sensitivity of 78.5% (detecting

543 of the 692 LC-MS/MS positives). Unfortunately, "21% of EIA for opiates show false negative results." The authors conclude, "LC-MS/MS methods are superior in terms of sensitivity and number of compounds that can be screened, making this a better method for use in pain management" (Snyder et al., 2017).

A retrospective chart review was conducted by Vopat et al. (2020) for a community-based practice, where 166 patients were examined. Motivated by studies that showed increases in post-operative orthopedic complications associated with pre-operative opioid use, the authors set out to determine whether urine drug screening (UDS) could be an effective screening tool for detecting opioid and illicit drugs prior to joint arthroplasty procedures. In the review, positive UDS results were compared to self-reported history of prescribed opioids. The authors demonstrated using four drug panels that of the 166 patients screened with UDS, 64 (38.6%) tested positive for opiate/opioids, while seven (4.2%) tested positive for amphetamines, six (3.6%) for cannabinoids, and two (1.2%) for other drugs, with one participant testing positive across multiple panels. However, it was also admitted that the study may have limited power, given that the population came from a single clinic with a limited number of cases. The narrow detection time of using urine detection screening also presents an issue; for example, drugs such as oxycodone may not be detected if administered more than three days before testing, leading to underestimation. Moreover, the data was not normalized for duration and dosage of opioid use, which are believed to contribute to clinical outcomes. However, the authors ultimately concluded that "With a significant number of patients testing positive for opioids without evidence of a previous prescription, UDS may be beneficial for initial risk assessment for patients undergoing JA procedures" (Vopat et al., 2020).

Palamar et al. (2019) completed research to determine the effectiveness of hair versus urine testing to detect or validate drug use. Data from 532 adults was used in this study. All participants reported using heroin or a nonmedical prescription opioid in the past month. Urine samples were obtained from all participants and almost 80% of participants provided hair samples. "Compared to hair testing, urine testing was able to confirm higher proportions of self-reported use of heroin/opioids (85.5% vs. 80.9%), marijuana (73.9% vs. 22.9%), benzodiazepines (51.3% vs. 15.1%), and methadone (77.0% vs. 48.7%), while hair testing was more likely to detect reported cocaine use (66.3% vs. 48.0%) (Ps<.01). Compared to hair testing, urine testing was more likely to detect unreported use of marijuana (11.3% vs. 0.9%), and benzodiazepines (14.4% vs. 5.4%), and hair testing was more likely to detect unreported use of cocaine (27.0% vs. 5.8%) and oxycodone (19.7% vs. 1.4%)" (Palamar et al., 2019). When used together, hair testing increased the detection of cocaine and/or oxycodone use from 14% to 22%. This is not surprising as cocaine is metabolized very quickly and may be undetectable in urine within hours to a few days depending on use (Nelson & Odujebe, 2023).

Böttcher et al. (2019) evaluated the analytical findings in oral fluid after oral fluid heroin intake. The study used 6-acetylmorphine (6-AM) as the target analyte. 2814 samples from 1875 patients were included. At a cut-off of 1 ng/mL "neat" (undiluted) oral fluid, 406 samples contained at least one opiate in the drug screening. 314 of these samples had a measured 6-AM concentration of ≥1 ng/mL. The authors also noted that the positive rates for opiates in oral fluid and urine were identical at 13.5% (in similar populations of patients). The authors concluded that 6-AM "…makes OF drug testing for detecting heroin use more effective than urine drug testing when using highly sensitive mass spectrometry methods" (Böttcher et al., 2019).

A study by Krasowski et al. (2020) used data from a College of American Pathologists survey on urine drug testing and screening proficiency to greater understand the strengths and weaknesses of immunoassays in drug testing. The authors note that there is a strong clinical interest for urine drug

testing, and that both opiate and amphetamine immunoassays were highly variable regarding cross-reactivity for drugs other than the actual assay calibrator. The authors also found that "urine drug testing availability does not parallel prevailing patterns of drug prescribing and abuse patterns. In particular, specific immunoassays for synthetic opioids and a lower positive cutoff for opiate immunoassays may be underused, whereas immunoassays for barbiturates, methadone, propoxyphene, and phencyclidine may be overused" (Krasowski et al., 2020).

Argoff et al. (2018) published a consensus report regarding "urine drug monitoring (UDM) in patients with chronic pain who are prescribed opioids." It is important to note that this publication was sponsored by major toxicology laboratories. The specialists convened were "an interdisciplinary group of clinicians with expertise in pain, substance use disorders, and primary care". They have issued recommendations based on their review of relevant literature, existing guidelines, and their clinical experiences in UDM. Their relevant recommendations are listed below:

- "Use definitive UDM testing (e.g., with GC-MS, LC-MS, or LC-MS/MS) as the most accurate method for assessing baseline opioid use and opioid misuse in almost all patients with chronic pain being considered for opioids as well as for ongoing monitoring of patients receiving opioids for chronic pain, unless presumptive testing is required by institutional or payer policies." The guideline acknowledges that "The recommendations in this consensus are intended to be considered together with practical clinical and payer concerns. When required by payers and institutions, immunoassays may be sufficient for monitoring low-risk patients, particularly when clinicians and patients engage in open communication."
- "Perform UDM at baseline in patients prescribed opioids for chronic pain. During ongoing monitoring, perform UDM at least annually for low-risk patients, two or more times per year for moderate-risk patients, and three or more times per year for high-risk patients. Additional monitoring can be performed at any risk level as frequently as necessary according to clinical judgment (Argoff et al., 2018)".

Guidelines and Recommendations

Several organizations recognize the benefit of drug screening/testing for the identification and management of drug misuse and abuse; however, standard guidelines for who should be tested, what test should be used, and how frequently testing should occur, are lacking.

Centers for Disease Control and Prevention (CDC)

In 2022, the CDC updated guidelines for prescribing opioids for pain (Dowell et al., 2022). Within the guidelines, the CDC recommends that clinicians should consider toxicology testing for care management. The CDC also recommends that "when prescribing opioids for subacute or chronic pain, clinicians should consider the benefits and risks of toxicology testing to assess for prescribed medications as well as other prescribed and nonprescribed controlled substances (recommendation category: B, evidence type: 4)." The CDC states that "toxicology testing should not be used in a punitive manner buts should be used in the context of other clinical information to inform and improve patient care," but specifically for urine drug testing, "urine toxicology tests do not provide accurate information about how much or what doses of opioids or other drugs a patient took…Detailed considerations for interpretation of urine toxicology test results, including which tests to order and expected results, drug detection time in urine, and drug metabolism, have been published previously" (Dowell et al., 2022).

Concerning the frequency of urine drug testing, in their 2016 guideline, the CDC stated, "While experts

agreed that clinicians should use urine drug testing before initiating opioid therapy for chronic pain, they disagreed on how frequently urine drug testing should be conducted during long-term opioid therapy. Most experts agreed that urine drug testing at least annually for all patients was reasonable. Some experts noted that this interval might be too long in some cases and too short in others, and that the follow-up interval should be left to the discretion of the clinician. Previous guidelines have recommended more frequent urine drug testing in patients thought to be at higher risk for substance use disorder. However, experts thought that predicting risk prior to urine drug testing is challenging and that currently available tools do not allow clinicians to reliably identify patients who are at low risk for substance use disorder" (Dowell et al., 2016).

The CDC also published a guideline "Quality Improvement and Care Coordination: Implementing the CDC Guideline for Prescribing Opioids for Chronic Pain" to provide guidance to healthcare systems and practice leaders. In it, the CDC details specific procedures to take when "unexpected results" appear.

- When the UDT is negative for a prescribed opioid, the CDC recommends repeating the test "using chromatography" and to specify the drug of interest.
- When the UDT is positive for a non-prescribed opioid, benzodiazepines, or illegal drugs, the CDC recommends repeating the UDT regularly.
- When the urine sample has a creatinine level of <2-3 mmol/L or < 20 mg/dL, the CDC recommends repeating the UDT.
- When the urine sample is cold, the CDC recommends repeating the UDT" (CDC, 2018b).

The CDC is currently working to update its resources and materials in accordance with the newly released *2022 CDC Clinical Practice Guideline for Prescribing Opioids for Pain*.

American Academy of Family Physicians (AAFP)

The AAFP published in 2019 recommendations concerning ordering and interpreting urine drug tests. They state, "Several federal and state regulations have been enacted that recommend or require urine drug testing in patients receiving long-term opioid therapy. Similar guidance may apply to patients receiving long-term benzodiazepine or stimulant therapy" (Kale, 2019). They state that the frequency of urine drug testing depends on individual risk factors and is ultimately left to the attending physician; however, they do state a recommended frequency for urine drug testing given in the table below:

Recommended Frequency for Urine Drug Testing (Kale, 2019)	
Level of misuse risk	Frequency of testing
Low (no risk factors)	Every 6 to 12 months
Moderate	Every 3 to 6 months
High (mental health disorder, substance use disorder, prior opioid misuse, aberrant behavior*) or opioid dosage >120 morphine milligram equivalents	Every 1 to 3 months
***Aberrant behavior includes, but is not limited to, lost prescriptions, multiple requests for early refills, opioid prescriptions from multiple physicians, unauthorized dose escalation, and apparent intoxication.**	

They state the following clinical recommendation: "Urine drug testing can be used to monitor compliance with prescribed therapy and detect the use of nonprescribed and illicit substances, especially opioids, benzodiazepines, and heroin".

In 2020, the AAFP provided a clinical preventive service recommendation on screening for opioid use disorder, stating that "The AAFP recommends that clinicians selectively screen and refer adults aged 18 years and older to OUD treatment after weighing the benefits and harms of screening and treatment. Clinicians should consider all benefits and harms including health, social, and legal outcomes. Screening programs should only be implemented if services for accurate diagnosis, effective treatment, and psychosocial supports can be offered or referred". This recommendation falls under the category of grade C, or the recommendation provides "at least moderate certainty that the net benefit is small" (AAFP, 2020).

Federation of State Medical Boards (FSMB)

The FSMB indicates in their Guidelines for Chronic Use of Opioid Analgesics policy that for patients being prescribed opioids for chronic pain management that the initial workup should include a system review and relevant physical examination, as well as laboratory investigations as indicated (FSMB, 2017). They also note the utility of periodic and unannounced testing for monitoring adherence to the patient's treatment plan and to detect non-prescribed drugs. Regarding frequency of testing, "Patients being treated for addiction should be tested as frequently as necessary to ensure therapeutic adherence, but for patients being treated for pain, clinical judgment trumps recommendations for frequency of testing" (FSMB, 2017).

Additionally, relative to how testing should be performed, the Federation of State Medical Boards notes that POC tests have significant limitations in both sensitivity and specificity, and therefore "the use of point of care testing for the making of more long term and permanent changes in management of people with the disease of addiction and other clinical situations may not be justified until the results of confirmatory testing with more accurate methods … are obtained." They do state, "Urine may be the preferred biologic specimen for testing because of its ease of collection and storage and the cost-effectiveness of such testing. When such testing is conducted as part of pain treatment, forensic standards are generally not necessary and not in place" (FSMB, 2017). They also note that initial testing could be done using immunoassays and followed up by a more specific technique, such as GC/MS or other chromatography-based technique. They highlight the importance of knowing specific drug and metabolites, "not just the class of drug" for the pain management.

American Academy of Pain Medicine

The AAPM notes that "urine and/or blood drug screening… may be helpful in ruling out the issue of diversion," along with other non-testing actions. They also note that "when appropriate, the patient should undergo a baseline drug screening exam." They highlight the importance of random urine drug screening for the ongoing monitoring of patient compliance to the treatment plan (AAPM, 2013).

The AAPM also co-sponsored guidelines with the American Association for Clinical Chemistry in 2018. These guidelines by Langman and Jannetto (2018) are shown below.

American Association for Clinical Chemistry (AACC)

In 2017, the AACC published their guidelines titled *Using Clinical Laboratory Tests to Monitor Drug Therapy in Pain Management Patients* (Jannetto et al., 2017). These guidelines were reaffirmed in 2018 and co-sponsored by the AAPM (Langman & Jannetto, 2018). The AACC lists medications in tiers to guide ordering of tests. Tier 1 is "routine monitoring" and includes frequently abused drugs as well as drugs frequently prescribed to pain management patients. Benzodiazepines, amphetamines, and barbiturates are in this tier. Anticonvulsants and antidepressants fall in tier 2, which is as follows: "High-

risk patients with known history of abuse for this medication or prevalence of drug use is endemic to local region, risky polypharmacy, multiple providers, or if prescribed and patient shows lack of efficacy or toxicity" (Jannetto et al., 2017). Antipsychotics fall in tier 3: which should be ordered "as clinically indicated."

The NACB [AACC] lists their recommendations with a grade for the quality of evidence as well as the strength of recommendation. An A represents a strong recommendation, a B is moderate recommendation, and C is a recommendation against. For the quality of evidence, an "I" represents "consistent results from well-designed, well-conducted studies in representative populations" whereas an "II" means "Evidence is sufficient to determine effects, but the strength of the evidence is limited by the number, quality, or consistency of the individual studies; generalizability to routine practice; or indirect nature of the evidence." The NACB's recommendations are as follows (Jannetto et al., 2017; Langman & Jannetto, 2018):

- "Testing biological specimens for drugs/drug metabolites is recommended and effective for detecting the use of relevant over-the-counter, prescribed and non-prescribed drugs, and illicit substances in pain management patients. Laboratory testing does not specifically identify most other outcomes, but should be used in conjunction with additional information to detect other outcomes in pain management patients. Strength of Recommendation: A; Quality of Evidence: I"
- "More frequent laboratory testing is recommended for patients with a personal or family history of substance abuse, mental illness, evidence of aberrant behavior, or other high-risk characteristics. Strength of Recommendation: A; Quality of Evidence: II"
- "Laboratory testing is recommended to identify the use of relevant over-the-counter medications, prescribed and non-prescribed drugs, and illicit substances in pain management patients. However, it does not effectively identify all non-compliance with the prescribed regimen. No single monitoring approach provides adequate information about the pattern or dose of patient drug use. Safest prescribing habits should include a combination of tools and laboratory test results to correctly detect outcomes. Strength of recommendation: A; Quality of evidence: III (pain management population), II (substance abuse disorder monitoring population)"
- "Laboratory testing is more effective than other physician tools for the detection of relevant over-the-counter, prescribed and non-prescribed drugs, and illicit substances in pain management patients and should be used routinely to monitor compliance. Strength of recommendation: A; Quality of evidence: II"
- "Urine testing is recommended for the detection of relevant over-the-counter medications, prescribed and nonprescribed drugs, and illicit substances in pain management patients. Strength of recommendation: B; Quality of evidence: II"
- "Based on level II evidence, baseline drug testing should be performed prior to initiation of acute or chronic controlled substance therapy. In addition, random drug testing should be performed at a minimum of one to two times a year for low-risk patients (based on history of past substance abuse/addiction, aberrant behaviors, and opioid risk screening criteria), with increasing frequency for higher-risk patients prescribed controlled substances. Strength of Recommendation: A; Quality of Evidence: II"
- "Serum or plasma is an acceptable alternate matrix for the detection of relevant over-the-counter medications, prescribed and non-prescribed drugs, and illicit substances in pain management patients with end-stage renal failure (anuria). For dialysis patients, the blood (serum/plasma) should be collected prior to dialysis. Oral fluid testing can also be used for selected drugs (e.g. amphetamine, benzodiazepines, buprenorphine, tetrahydrocannabinol, cocaine, codeine, hydrocodone, hydromorphone, methadone, morphine, oxycodone, and oxymorphone). Strength of recommendation: A; Quality of evidence: III"

- "While definitive testing is recommended and preferred, urine immunoassays performed on laboratory-based analyzers offer some clinical utility to detect the use of relevant over-the-counter medications, prescribed and non-prescribed drugs, and illicit substances in pain management patients. However, physicians using immunoassay-based tests (especially amphetamine, benzodiazepine, and opiate immunoassays) must reference the package insert if testing in the physician's office or consult with laboratory personnel to evaluate the assay's capabilities and limitations for detecting specific medications within a drug class to prevent incorrect interpretation and to determine when additional testing is necessary. Strength of Recommendation: B; Quality of Evidence: II"
- "Qualitative definitive tests should be used over immunoassays since they are more effective at identifying relevant over-the-counter medications, prescribed and non-prescribed drugs, and illicit substances in pain management patients. Strength of Recommendation: A; Quality of Evidence: II"
- "Qualitative definitive tests should be used when possible over immunoassays for monitoring use (compliance) to relevant over-the-counter medications, prescribed and non-prescribed drugs, and illicit substances in pain management patients due to their superior sensitivity and specificity. Strength of Recommendation: A; Quality of Evidence: II"
- "POC (oral/urine) qualitative presumptive immunoassays offer similar performance characteristics to laboratory-based immunoassays and can detect some over-the-counter medications, prescribed and non-prescribed drugs, and illicit substances in pain management patients. However, physicians using POC testing must reference the POC package insert and/or consult laboratory personnel to accurately determine the assay's capabilities (especially amphetamine, benzodiazepine, and opiate immunoassays) and understand the limitations for detecting specific medications within a drug class to prevent incorrect assumptions or interpretation and to determine when additional testing is necessary. Strength of Recommendation: B; Quality of Evidence: II"
- "Qualitative immunoassay drug testing prior to prescribing controlled substances can be used to identify some illicit drug use and decrease adverse outcomes in pain management patients. Strength of Recommendation: B; Quality of Evidence: II"
- "Random urine testing for relevant over-the-counter medications, prescribed and non-prescribed drugs, and illicit substances is recommended to detect outcomes in pain management patients. Strength of Recommendation: A; Quality of Evidence: III (pain management population), II (substance abuse disorder monitoring population)"
- "Appropriately performed and interpreted urine POC immunoassay testing can be cost-effective for detecting use or inappropriate use of some over-the-counter medications, prescribed and non-prescribed drugs, and illicit substances in pain management patients. Strength of Recommendation: B; Quality of Evidence: II"
- "Firstline definitive testing (qualitative or quantitative) is recommended for detecting the use of relevant over-the-counter medications, prescribed and non-prescribed drugs, and illicit substances in pain management patients. Strength of recommendation: A; Quality of evidence: II"
- "Recommend definitive testing for any immunoassay (laboratory-based or POC) result that isn't consistent with the clinical expectations in a pain management patient. Strength of recommendation: A; Quality of evidence: III"
- "Quantitative definitive urine testing is not more useful at detecting outcomes in pain management patients compared to qualitative definitive urine testing. Furthermore, quantitative definitive urine testing should not be used to evaluate dosage of administered drug or adherence to prescribed dosage regimen. However, quantitative urine definitive testing is recommended to identify variant drug metabolism, detect pharmaceutical impurities, or metabolism through minor

routes. Quantitative results may also be useful in complex cases to determine the use of multiple opioids, confirm spiked samples, and/or rule out other sources of exposure (e.g. morphine from poppy seeds). Strength of recommendations: A; Quality of evidence: II"

- "The use of lower limit-of-detection cutoff concentrations can be more effective to detect use (either partial or full compliance) or the lack of use of relevant over-the-counter medications, prescribed and non-prescribed drugs, and illicit substances in pain management patients, especially those taking lower dosages. Strength of Recommendation: B; Quality of Evidence: II" (Jannetto et al., 2017; Langman & Jannetto, 2018).

American Pain Society/American Academy of Pain Medicine

The American Pain Society and American Academy of Pain Medicine joint guidelines panel released their opioid treatment guidelines titled *Clinical Guidelines for the Use of Chronic Opioid Therapy in Chronic Non-cancer Pain* in 2009. They addressed the monitoring of controlled substances use via UDT as part of a chronic opioid treatment (COT) program. The authors recommend periodic urine drug screening and suggest that random urine drug screens may be more informative than scheduled or routine testing. The guideline section on monitoring (Section 5) states:

- "5.1: Clinicians should reassess patients on COT periodically and as warranted by changing circumstances. Monitoring should include documentation of pain intensity and level of functioning, assessments of progress toward achieving therapeutic goals, presence of adverse events, and adherence to prescribed therapies (strong recommendation, low-quality evidence).
- 5.2: In patients on COT who are at high risk or who have engaged in aberrant drug-related behaviors, clinicians should periodically obtain urine drug screens or other information to confirm adherence to the COT plan of care (strong recommendation, low-quality evidence).
- 5.3: In patients on COT not at high risk and not known to have engaged in aberrant drug-related behaviors, clinicians should consider periodically obtaining urine drug screens or other information to confirm adherence to the COT plan of care (weak recommendation, low-quality evidence). Clinicians should periodically reassess all patients on COT. Regular monitoring of patients once COT is initiated is critical because therapeutic risks and benefits do not remain static" (Chou et al., 2009).

The American Pain Society guidelines state that for individuals at low risk for adverse outcomes, quarterly or semi-annual monitoring is sufficient. The risk for abuse may be measured using standard tools, such as the Screener and Opioid Assessment for Patients with Pain (SOAPP) and the Opioid Risk Tool. These types of tools may help clinicians assess the suitability of long-term opioid therapy for chronic pain patients and may help differentiate those patients who require more clinician monitoring while on long-term opioid therapy. Both tools may be self-administered at or prior to an office visit, or completed as part of an interview with a nurse, physician or psychologist (Chou et al., 2009).

American Society of Interventional Pain Physicians (ASIPP)

ASIPP issued evidence-based clinical practice guidelines to improve the quality of care through responsible opioid prescribing in non-cancer pain. They have described evidence assessment followed in Part One of the guidelines and the recommended guidance in Part Two.

ASIPP provides 11 recommendations including drug cut-offs and detection limits for drugs of abuse, drug cross-reactants, guidance on interpretation of unexpected results for urine drug testing and urine drug testing algorithm. In their algorithm, ASIPP proposes to perform baseline assessment of the patient with chronic pain using POC immunoassay. Then, depending on the result to continue either compliance monitoring with random POC immunoassay in 1-3 months if initial results were appropriate or explained, followed-up with random testing in 6-12 months if the result remains appropriate. In the case when inappropriate or unexplained results are obtained, confirmatory testing is proposed with repeat urine drug testing in one month or next appointment (Manchikanti et al., 2012).

In their recommendation 1D, level of evidence good, ASIPP states: "Urine drug testing (UDT) must be implemented from initiation along with subsequent adherence monitoring to decrease prescription drug abuse or illicit drug use when patients are in chronic pain management therapy." Additionally, they state, "In order to reduce prescription drug abuse and doctor shopping, adherence monitoring by UDT and PMDPs provide evidence that is essential to the identification of those patients who are non-compliant or abusing prescription drugs or illicit drugs." Level of evidence is fair (Manchikanti et al., 2012).

A 2017 update from ASIPP reaffirms the use of urine drug testing and monitoring programs when taking the initial steps towards opioid therapy, captured below.

"1. Comprehensive assessment and documentation. (Evidence: Level I; Strength of Recommendation: Strong)
2. Screening for opioid abuse to identify opioid abusers. (Evidence: Level II-III; Strength of Recommendation: Moderate)
3. Utilization of prescription drug monitoring programs (PDMPs). (Evidence: Level I-II; Strength of Recommendation: Moderate to strong)
4. Utilization of urine drug testing (UDT). (Evidence: Level II; Strength of Recommendation: Moderate)
5. Establish appropriate physical diagnosis and psychological diagnosis if available. (Evidence: Level I; Strength of Recommendation: Strong)
6. Consider appropriate imaging, physical diagnosis, and psychological status to collaborate with subjective complaints. (Evidence: Level III; Strength of Recommendation: Moderate)
7. Establish medical necessity based on average moderate to severe (? 4 on a scale of 0 – 10) pain and/or disability. (Evidence: Level II; Strength of Recommendation: Moderate)
8. Stratify patients based on risk. (Evidence: Level I-II; Strength of Recommendation: Moderate)
9. Establish treatment goals of opioid therapy with regard to pain relief and improvement in function. (Evidence: Level I-II; Strength of Recommendation: Moderate)
10. Obtain a robust opioid agreement, which is followed by all parties. (Evidence: Level III; Strength of Recommendation: Moderate) (Manchikanti et al., 2017)".

Monitoring may also continue for adherence and side effects, extending through the final phases:

"19. Monitor for adherence, abuse, and noncompliance by UDT and PDMPs. (Evidence: Level I-II; Strength of Recommendation: Moderate to strong)
20. Monitor patients on methadone with an electrocardiogram periodically. (Evidence: Level I; Strength of Recommendation: Strong).
21. Monitor for side effects including constipation and manage them appropriately, including discontinuation of opioids when indicated. (Evidence: Level I; Strength of Recommendation: Strong)
iv. Final Phase
22. May continue with monitoring with continued medical necessity, with appropriate outcomes. (Evidence: Level I-II; Strength of Recommendation: Moderate)

23. Discontinue opioid therapy for lack of response, adverse consequences, and abuse with rehabilitation. (Evidence: Level III; Strength of Recommendation: Moderate) (Manchikanti et al., 2017)".

Washington State Agency Medical Directors' Group (AMDG)

The Washington State AMDG published an Interagency Guideline on opioid dosing for chronic non-cancer pain. This guideline and related expert commentary support low-risk individuals having UDT up to once per year, moderate risk up to two per year, high risk individuals up to three to four tests per year, and individuals exhibiting aberrant behaviors should be tested at the time of the office visit (AMDG, 2015).

Supplemental guidance on prescribing opioids for postoperative pain was published by the AMDG in 2018. Specific opioid testing methods are not mentioned in these guidelines (AMDG, 2018).

Wisconsin Worker's Compensation Patient Care

Wisconsin's Worker's Compensation program recommends for any worker's compensation patient who will need opioid treatment for a period of more than 90 days, that the treating physician should follow these guidelines and or consider referral to a Pain Management specialist. In their document, they state that "urine drug screening before starting chronic opioid therapy is imperative" to verify that patient is not using illegal substances. In addition, according to their guidelines, compliance monitoring is mandatory for all patients on chronic opioid therapy with several tools including urine drug screen for the first visit and with aberrant behavior and unannounced urine drug screens thereafter (DWD, 2013).

American Society of Addiction Medicine (ASAM)

ASAM states quantification (assessing specific concentration of a drug) should not be used to determine adherence with a specific dosage or formulation regimen. There are, however, specific reasons for obtaining quantitative data. For example, quantification can help a clinician decide why the other opioids are present. Serial creatinine-corrected quantitative values can help the clinician distinguish cessation of drug use from continued drug excretion from ongoing drug use. Finally, the guidelines note that state laws may also guide testing decisions (ASAM, 2013).

In 2017, the ASAM recommended drug testing as "an important supplement to self-report because patients may be unaware of the composition of the substance(s) they have used" (Jarvis et al., 2017). They also recommend to not rely on the SAMHSA-5 panel as a routine drug panel. ASAM states that urine testing for amphetamines and benzodiazepines may be helpful when assessing potential use. The society also emphasizes that the results must be carefully analyzed due to specificity limitations in both immunoassays.

With regards to general testing, ASAM recommends random, unannounced testing as opposed to scheduled ones. They recommend, "presumptive testing should be a routine part of initial and ongoing patient assessment." Concerning definitive drug testing, they recommend, "Definitive testing techniques should be used whenever a provider wants to detect specific substances not identified by presumptive methods, quantify levels of the substance present, and refine the accuracy of the results. Definitive testing should be used when the results inform clinical decisions with major clinical or nonclinical implications for the patient (e.g. treatment transition, changes in medication therapies, changes in legal status)" (Jarvis et al., 2017). ASAM also considers GC/MS and LC-MS testing for confirmation of a presumptive positive test. For patients in substance abuse treatment, ASAM recommends frequent random testing (at least weekly) initially. Once the patient is stable in treatment, then the frequency can

decrease (to at least monthly).

New York State Office of Addiction Services and Supports (OASAS)

The OASAS published guidelines on toxicology testing during treatment for substance use disorders. The guidelines specify that toxicology testing may include urine, blood, breath, oral fluid, sweat, and hair, but note that urine testing is the most common and validated matrix.

The guidelines outline when toxicology testing should be completed. Toxicology testing should be used when clinically indicated, such as in circumstances of request, intake/admission, and during treatment, to determine which substances have been used recently and to guide further clinical decision making and testing. It can also be used in situations of testing drug court participants, and in opioid treatment programs, for which the guidelines indicate additional inclusion of "qualitative indicators of treatment progress, such as how the patient is functioning in their personal and/or professional life, to determine patient stability for more flexible take-home dosing." The guidelines further state "Toxicology testing is designed to identify whether a substance was taken within a specific time period. It should be used in conjunction with self-report and clinical assessment to obtain a full clinical picture" and "Substances should be included only if the toxicology tests have a reasonable degree of sensitivity and specificity and therefore can inform clinical care usefully beyond self-report, collateral report, and clinical evaluation." (OASAS, 2023).

Texas Pain Society

The Texas Pain Society released detailed guidelines concerning urine drug testing (UDT) and its use in the practice of pain management. They do not recommend a prescribed regimen of UDT but rather leave it to the discretion of the physician. They do recommend random UDT over scheduled UDT. Concerning what should be included in a UDT, "Elements of UDT may include specific gravity, temperature at the time of sample collection, pH, creatinine concentration, and mass spectroscopic confirmatory testing for the following agents: opioids (fentanyl, oxycodone, oxymorphone, tramadol, methadone, hydrocodone, hydromorphone, morphine, codeine, propoxyphene, meperidine, buprenorphine, tapentadol, 6-mono-acetyl morphine [6-MAM])…"(Owen et al., 2012). Concerning the frequency of conducting UDTs, they recommend 1-2 tests per year for low-risk patients; 3-4 tests per year for moderate-risk patients; and "4 [per year] or every month, office visit, or every drug refill" for high-risk patients.

2014 Annals of Internal Medicine Review

In 2014 Nuckols and colleagues released an extensive review of guidelines on prescribing and monitoring opioids from more than ten different societies and organizations in the *Annals of Internal Medicine*. No consensus concerning urine drug monitoring or testing was noted across all guidelines; in fact, the APS-AAPM noted to use UDT only "if risk is high; consider otherwise." The NOUGG recommends that, if UDT is used, to consider pros and cons (expert consensus). The Colorado Division of Workers Compensation requires mandatory UDT. The VA/DoD and ASIPP uses UDT to establish a baseline followed by random testing during treatment whereas the ACOEM and UMHS uses UDT to establish a baseline followed by either a minimum of quarterly testing or annual testing, respectively (Nuckols et al., 2014).

Substance Abuse and Mental Health Services Administration (SAMHSA)

These guidelines are for the certification for opioid treatment programs (OTPs). OTPs require certification before they can dispense opioids to treat opioid addiction. SAMHSA recommends opioids, methadone, amphetamines, cocaine, and benzodiazepines at a minimum be tested before admission to any opioid treatment program. Testing is not limited to these classes of drugs and may vary; any inclusion of other drugs for testing "should be determined by community drug use patterns or individual medical indications" (SAMHSA, 2015).

SAMHSA federal guidelines for opioid treatment programs were updated in 2015. These guidelines state that "It is strongly recommended that benzodiazepines, barbiturates, and alcohol (using the ethyl glucuronide test) be included in drug screening and testing panels (SAMHSA, 2015, 2023)." The guidelines also state that "OTPs often perform onsite point of collection (POC) tests using sensitive and automated immunoassay (IA) technologies that screen urine or oral fluid samples for a relatively narrow range of drug classes (e.g. amphetamines, barbiturates, benzodiazepines, opioids) and a limited number of specific drugs. POC tests such as IAs have a place in clinical decision making, but are not by themselves adequate to satisfy the regulatory requirements for drug use testing services" (SAMHSA, 2015).

In 2020, SAMHSA published guidelines regarding use of oral fluid for federal workplace drug testing programs. In it, they remarked that "The Department believes that collecting and testing oral fluid specimens according to the requirements in these Guidelines is an efficient means to detect illicit drug use and ensures that the oral fluid test results are forensically and scientifically supportable." SAMHSA writes that several reasons demanded the need for regulation of oral fluid testing, such as the need to decrease invalid urine tests. SAMHSA writes that an oral fluid specimen may be used for the following reasons: "a federal agency applicant/preemployment test, a random test, a reasonable suspicion/cause test, a post-accident test, a return to duty test, or a follow-up test" (SAMHSA, 2020).

American Association for the Treatment of Opioid Dependence Inc. (AATOD)

The AATOD recommends cessation of benzodiazepines before admission to an opioid treatment program (OTP). Gradually tapering off to a lower dose is also acceptable, but benzodiazepine use must be addressed prior to an OTP admission. The AATOD recommends toxicology screening for benzodiazepines, as well as routine checks of each state's Prescription Monitoring Drug Program. Confirmatory testing may also be used (AATOD, 2017).

Department of Health and Human Services (HHS)

The HHS has provided guidelines on Federal Workplace Drug Testing Programs. Federal agencies must comply with these guidelines by October 10, 2023. Each specimen must be tested for marijuana and cocaine. With regards to validity tests, the HHS rule states that "an HHS-certified laboratory is authorized to perform additional drug and/or specimen validity tests on a case-by case basis as necessary to provide information that the [Medical Review Officer] would use to report a verified drug test… an HHS-certified laboratory is not authorized to routinely perform additional drug and/or specimen validity tests at the request of an MRO without prior authorization from the Secretary or designated HHS representative, with the exception of the determination of d,l stereoisomers of amphetamine and methamphetamine." Additional drugs may be tested if the testing is done under reasonable suspicion or post-accident on a case-by-case approval basis. An adulterated specimen is defined as one that "has been altered, as evidenced by test results showing either a substance that is not a normal constituent for that type of specimen or showing an abnormal concentration of a normal constituent (*e.g.,* nitrite in urine)" (HHS, 2023).

Regarding the tests that should be conducted on an oral fluid specimen, a federal agency

> "(a) Must ensure that each specimen is tested for marijuana and cocaine as provided in the drug testing panel described under Section 3.4;
> (b) Is authorized to test each specimen for other Schedule I or II drugs as provided in the drug testing panel;
> (c) Is authorized upon a Medical Review Officer's request to test an oral fluid specimen to determine specimen validity using, for example, a test for a specific adulterant;
> (d) Is authorized to test each specimen for one or more biomarkers as provided in the biomarker testing panel described under Section 3.4; and
> (e) If a specimen exhibits abnormal characteristics (*e.g.,* unusual odor or color, semi-solid characteristics), causes reactions or responses characteristic of an adulterant during initial or confirmatory drug tests (*e.g.,* non-recovery of internal standard, unusual response), or contains an unidentified substance that interferes with the confirmatory analysis, then additional testing may be performed."

The rule also states that a federal agency may collect an oral fluid specimen under the following circumstances:

> "(a) Federal agency applicant/pre-employment test;
> (b) Random test;
> (c) Reasonable suspicion/cause test;
> (d) Post accident test;
> (e) Return to duty test; or
> (f) Follow up test."

Section 3.4 refer to drug and biomarker test analytes and cutoffs for undiluted (neat) oral fluids, and a screenshot is included below.

Initial test analyte	Initial test cutoff [1]	Confirmatory test analyte	Confirmatory test cutoff
Marijuana (THC) [2]	4 ng/mL [3]	THC	2 ng/mL.
Cocaine/Benzoylecgonine	15 ng/mL	Cocaine	8 ng/mL.
		Benzoylecgonine	8 ng/mL.
Codeine/Morphine	30 ng/mL	Codeine	15 ng/mL.
		Morphine	15 ng/mL.
Hydrocodone/Hydromorphone	30 ng/mL	Hydrocodone	15 ng/mL.
		Hydromorphone	15 ng/mL.
Oxycodone/Oxymorphone	30 ng/mL	Oxycodone	15 ng/mL.
		Oxymorphone	15 ng/mL.
6-Acetylmorphine	4 ng/mL [3]	6-Acetylmorphine	2 ng/mL.
Phencyclidine	10 ng/mL	Phencyclidine	10 ng/mL.
Amphetamine/Methamphetamine	50 ng/mL	Amphetamine	25 ng/mL.
		Methamphetamine	25 ng/mL.
MDMA [4]/MDA [5]	50 ng/mL.	MDMA	25 ng/mL.
		MDA	25 ng/mL.

[1] *For grouped analytes (i.e., two or more analytes that are in the same drug class and have the same initial test cutoff):*
Immunoassay: The test must be calibrated with one analyte from the group identified as the target analyte. The cross-reactivity of the immunoassay to the other analyte(s) within the group must be 80 percent or greater; if not, separate immunoassays must be used for the analytes within the group.
Alternate technology: Either one analyte or all analytes from the group must be used for calibration, depending on the technology. At least one analyte within the group must have a concentration equal to or greater than the initial test cutoff or, alternatively, the sum of the analytes present (i.e., equal to or greater than the laboratory's validated limit of quantification) must be equal to or greater than the initial test cutoff.
[2] An immunoassay must be calibrated with the target analyte, Δ-9-tetrahydrocannabinol (THC).
[3] *Alternate technology (THC and 6–AM):* The confirmatory test cutoff must be used for an alternate technology initial test that is specific for the target analyte (i.e., 2 ng/mL for THC, 2 ng/mL for 6–AM).
[4] Methylenedioxymethamphetamine (MDMA).
[5] Methylenedioxyamphetamine (MDA).

(HHS, 2023)

Furthermore, the Pain Management Best Practices Inter-Agency Task Force of HHS recognizes the importance of screening and monitoring in pain management in identifying and reducing the risk of

substance misuse, abuse, and overdose, as well as improving overall patient care. As such, they include a series of gaps in care and related recommendations regarding screening, including the following:

"GAP 1: Comprehensive screening and risk assessment of patients are time-consuming but vital for proper evaluation of their chronic pain conditions. Lack of sufficient compensation for time and payment for services have contributed to barriers in best practices for opioid therapy.

• RECOMMENDATION 1A: Encourage CMS and private payers to provide sufficient compensation for time and payment for services to implement the various screening measures (e.g., extensive history taking, review of medical records, PDMP query, urine toxicology screenings, when clinically indicated). These are vital aspects of risk assessment and stratification for patients on opioids and other medications.

• RECOMMENDATION 1B: Consider referral to pain, mental health, and other specialists, including addiction medicine-trained physicians when high-risk patients are identified.

GAP 2: UDTs are not consistently used as part of the routine risk assessment for patients on opioids.
• RECOMMENDATION 2A: Use UDTs as part of the risk assessment tools prior to the initiation of opioid therapy and as a tool for reevaluating risk, using the clinical judgment of the treatment team.

• RECOMMENDATION 2B: Clinicians should educate patients on the use of UDTs and their role in identifying both appropriate and potentially inappropriate use" (PMFT, 2019).

American Academy of Child and Adolescent Psychiatry (AACAP)

AACAP notes, "Toxicology screens are indicated for acute onset or exacerbations of psychosis when exposure to drugs of abuse cannot otherwise be ruled out. Genetic testing is indicated if there are associated dysmorphic or syndromic features" (McClellan & Stock, 2013).

World Federation of Societies of Biological Psychiatry (WFSBP)

The WFSBP states that drug screening (urine and blood) should be sought for schizophrenia patients as "presence of substance abuse or dependence is often not recognized and systematically assessed, especially if such a patient is seen during an acute psychotic episode" (WFSBP, 2015).

National Institute for Health and Care Excellence (NICE)

NICE notes that additional testing should be considered in adults to identify potential causes or co-morbidities, but the current guidelines do not mention the use of blood or urine testing, as once previously recommended. The following recommendations are the recommendations regarding underlying etiologies of epilepsy and testing:

- "In adults, assessment should include checking for the following modifiable factors that may increase the risk of a second seizure:
  - An underlying mental health problem (such as depression, anxiety, psychosis and alcohol or substance misuse
  - Vascular risk factors (for example, diabetes, hypertension, atrial fibrillation)
  - Sepsis"
- "Offer brain neuroimaging tests if an underlying structural cause is suspected"

- "Be aware of the possible underlying causes of status epilepticus, including hypoglycaemia, eclampsia, and alcohol withdrawal, which may need to be treated with additional medication" (NICE, 2022).

American Academy of Neurology (AAN)

The AAN states that "toxicology testing may be considered in children with status epilepticus, when no apparent etiology is immediately identified" (AAN, 2018). These guidelines were reaffirmed in January 22, 2022.

Department of Veterans Affairs/Department of Defense (VA/DOD)

In 2021, the VA/DOD issued recommendations surrounding the management of substance use disorders. In it, it was recommended that:

- "For patients in general medical and mental healthcare settings, we recommend screening for unhealthy alcohol use annually using the three-item Alcohol Use Disorders Identification Test-Consumption (AUDIT-C) or Single Item Alcohol Screening Questionnaire (SASQ) (Strength: Strong for)"
- "There is insufficient evidence to recommend for or against screening for drug use disorders in primary care to facilitate enrollment in treatment (Strength: Neither for nor against)" (DVA & DOD, 2021).

In 2022, the VA/DOD updated their clinical practice guidelines for opioid therapy for chronic pain. These guidelines recognize that "urine drug testing is an additional method of examining for patient substance misuse and adherence to the prescribed regimen" (DVA & DOD, 2022). The guidelines also state that "It is critical that the UDT and confirmatory testing be done in a timely, confidential, accurate, and easily available manner to assure the prescribers, patients, and public that safety, fairness, and trust are being addressed" (DVA & DOD, 2022). The VA/DOD also recognizes the three main types of UDTs: immunoassay, gas chromatography-mass spectrometry (GCMS) confirmatory testing, and liquid chromatography-mass spectrometry (LCMS) confirmatory testing). In their recommendation for risk mitigation, the VA/DOD "suggest urine drug testing for patients on long-term opioids (Strength: Weak for)" (DVA & DOD, 2022).

With respect to antepartum and peripartum use of alcohol, cigarettes, illicit drugs, and the like, these joint guidelines state "We recommend screening for use of tobacco and nicotine products, alcohol, cannabis, illicit drugs, and inappropriate use of prescription medication (Strength: Strong for)" (DVA & DOD, 2023).

Anxiety Disorders Association of Canada (ADAC)

The ADAC recommends urine toxicology as part of the patient's baseline investigations if warranted. This urine toxicology assessment applies to anxiety and other related disorders, which include "panic disorder, agoraphobia, GAD, selective mutism, separation anxiety disorder, SAD (social phobia), specific phobia, substance/medication-induced anxiety disorder, as well as anxiety disorder due to another medical condition or not elsewhere classified" (Katzman et al., 2014).

American Psychiatric Association, Practice Guidelines for the Psychiatric Evaluation of Adults, 3rd Edition (2016)

The Association acknowledges that urine toxicology may provide clues to substance abuse during an initial psychiatric evaluation (APA, 2016).

World Health Organization (WHO)

The WHO released an intervention guideline for mental, neurological, and substance use disorder in non-specialized health settings. The WHO states that urine testing may be considered to confirm abstinence and to "consider occasional urine testing to confirm non-use." Under the section concerning the investigation of chronic drug use, they state to consider using urine drug screens "for emergency cases, a urine drug screen should be conducted whenever intoxication, withdrawal, or overdose is suspected, especially in cases when the person is unable to convey what they have ingested" (WHO, 2016). The WHO lists the following substances as psychoactive substances: alcohol, benzodiazepines, opioids, tobacco, cocaine, methamphetamines, amphetamine-type stimulants, khat, cannabis, tramadol, "volatile" solvents, MDMA, and hallucinogens.

American College of Obstetricians and Gynecologists (ACOG)

ACOG states that additional research is needed to better understand the effects of universal urine screening on clinical outcomes and recommend validated verbal screening tools instead. ACOG acknowledges that urine drug testing has been used to identify substance abuse and should only be performed in compliance with state's laws and with patient consent. ACOG also lists the following recommendations:

- "Screening for substance use should be part of comprehensive obstetric care and should be done at the first prenatal visit in partnership with pregnant woman. Screening based only on factors, such as poor adherence to prenatal care or prior adverse pregnancy outcome, can lead to missed cases and may add to stereotyping and stigma. Therefore, it is essential that screening be universal."
- "Routine screening should rely on validated screening tools, such as questionnaires, including 4Ps, NIDA, Quick Screen, and CRAFFT (for women 26 years or younger) (ACOG, 2017).

ACOG explicitly states, "Routine urine drug screening is controversial for several reasons. A positive drug test result is not in itself diagnostic of opioid use disorder or its severity. Urine drug testing only assesses for current or recent substance use; therefore, a negative test does not rule out sporadic substance use… Health care providers should be aware of t heir laboratory's test characteristics and request that confirmatory testing with mass spectrometry and liquid or gas chromatography be performed as appropriate" (ACOG, 2017). This guideline was reaffirmed in 2021.

Society of Obstetricians and Gynaecologists of Canada (SOGC)

The SOGC recommends periodic drug screening for all pregnant women and all women of childbearing age (III-A). The recommended method of drug screening is a urine toxicology screen (II-2A); however, they state that prior to maternal drug toxicology testing is ordered that informed consent be obtained (III-B) (Wong et al., 2011).

Updated 2017 SOGC guidelines state that "When testing for substance use is clinically indicated, urine drug screening is the preferred method (II-2A)" (Ordean et al., 2017).

Canadian Paediatric Society (CPS)

In 2017, the CPS—in a position statement dealing with cannabis in Canada's children and youth—urged the following recommendation for healthcare providers: "Screen all children and youth for cannabis exposure and/or use and educate adolescents and families on the health risks and harms associated with cannabis" (Grant & Bélanger, 2017). This statement was reaffirmed February 24, 2023.

The CPS, within their 2018 guidelines, on ADHD in children and youth state, "Children with ADHD may also experience comorbid depressive symptoms, particularly as they approach adolescence and adulthood. There is increasing evidence of heterotypic continuity between these two conditions, suggesting they may represent the same underlying construct for some children. The validity of BD diagnosis, particularly when broadly defined, remains controversial in preadolescent children… There is an increase in SUDs as children with ADHD reach adolescence and adulthood. It is possible that substance use occurs as an attempt to self-medicate. The treatment of ADHD comorbid with a SUD is complicated by risks for misuse and diversion of prescription stimulants" (Bélanger et al., 2018). The CPS makes no statement regarding mode of testing or frequency of testing.

Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
80305	Drug test(s), presumptive, any number of drug classes, any number of devices or procedures; capable of being read by direct optical observation only (e.g., utilizing immunoassay [eg, dipsticks, cups, cards, or cartridges]), includes sample validation when performed, per date of service
80306	Drug test(s), presumptive, any number of drug classes, any number of devices or procedures; read by instrument assisted direct optical observation (eg, utilizing immunoassay [eg, dipsticks, cups, cards, or cartridges]), includes sample validation when performed, per date of service
80307	Drug test(s), presumptive, any number of drug classes, any number of devices or procedures; by instrument chemistry analyzers (eg, utilizing immunoassay [eg, EIA, ELISA, EMIT, FPIA, IA, KIMS, RIA]), chromatography (eg, GC, HPLC), and mass spectrometry either with or without chromatography, (eg, DART, DESI, GC-MS, GC-MS/MS, LC-MS, LC-MS/MS, LDTD, MALDI, TOF) includes sample validation when performed, per date of service

CPT	Code Description
0007U	Drug test(s), presumptive, with definitive confirmation of positive results, any number of drug classes, urine, includes specimen verification including DNA authentication in comparison to buccal DNA, per date of service Proprietary test: ToxProtect Lab/Manufacturer: Genotox Laboratories LTD
0011U	Prescription drug monitoring, evaluation of drugs present by LC-MS/MS, using oral fluid, reported as a comparison to an estimated steady-state range, per date of service including all drug compounds and metabolites Proprietary test: Cordant CORE™ Lab/Manufacturer: Cordant Health Solutions
0051U	Prescription drug monitoring, evaluation of drugs present by LC-MS/MS, urine, 31 drug panel, reported as quantitative results, detected or not detected, per date of service Proprietary test: UCompliDx Lab/Manufacturer: Elite Medical Laboratory Solutions, LLC (LDT)
0054U	Prescription drug monitoring, 14 or more classes of drugs and substances, definitive tandem mass spectrometry with chromatography, capillary blood, quantitative report with therapeutic and toxic ranges, including steady-state range for the prescribed dose when detected, per date of service Proprietary test: AssuranceRx Micro Serum Lab/Manufacturer: Firstox Laboratories, LLC
0079U	Comparative DNA analysis using multiple selected single-nucleotide polymorphisms (SNPs), urine and buccal DNA, for specimen identity verification Proprietary test: ToxLok™ Lab/Manufacturer: InSource Diagnostics
0082U	Drug test(s), definitive, 90 or more drugs or substances, definitive chromatography with mass spectrometry, and presumptive, any number of drug classes, by instrument chemistry analyzer (utilizing immunoassay), urine, report of presence or absence of each drug, drug metabolite or substance with description and severity of significant interactions per date of service Proprietary test: NextGen Precision™ Testing Lab/Manufacturer: Precision Diagnostics LBN Precision Toxicology, LLC
0093U	Prescription drug monitoring, evaluation of 65 common drugs by LC-MS/MS, urine, each drug reported detected or not detected Proprietary test: ComplyRX Lab/Manufacturer: Claro Labs
0227U	Drug assay, presumptive, 30 or more drugs or metabolites, urine, liquid chromatography with tandem mass spectrometry (LC-MS/MS) using multiple reaction monitoring (MRM), with drug or metabolite description, includes sample validation Proprietary Test: Comprehensive Screen Lab/Manufacturer: Aspenti Health
0328U	Drug assay, definitive, 120 or more drugs and metabolites, urine, quantitative liquid chromatography with tandem mass spectrometry (LC-MS/MS), includes specimen validity and algorithmic analysis describing drug or metabolite and presence or absence of risks for a significant patient-adverse event, per date of service Proprietary test: CareView360 Lab/Manufacturer: Newstar Medical Laboratories, LLC

CPT	Code Description
G0480	Drug test(s), definitive, utilizing (1) drug identification methods able to identify individual drugs and distinguish between structural isomers (but not necessarily stereoisomers), including, but not limited to GC/MS (any type, single or tandem) and LC/MS (any type, single or tandem and excluding immunoassays (e.g., IA, EIA, ELISA, EMIT, FPIA) and enzymatic methods (e.g., alcohol dehydrogenase)), (2) stable isotope or other universally recognized internal standards in all samples (e.g., to control for matrix effects, interferences and variations in signal strength), and (3) method or drug-specific calibration and matrix-matched quality control material (e.g., to control for instrument variations and mass spectral drift); qualitative or quantitative, all sources, includes specimen validity testing, per day; 1-7 drug class(es), including metabolite(s) if performed
G0481	Drug test(s), definitive, utilizing (1) drug identification methods able to identify individual drugs and distinguish between structural isomers (but not necessarily stereoisomers), including, but not limited to GC/MS (any type, single or tandem) and LC/MS (any type, single or tandem and excluding immunoassays (e.g., IA, EIA, ELISA, EMIT, FPIA) and enzymatic methods (e.g., alcohol dehydrogenase)), (2) stable isotope or other universally recognized internal standards in all samples (e.g., to control for matrix effects, interferences and variations in signal strength), and (3) method or drug-specific calibration and matrix-matched quality control material (e.g., to control for instrument variations and mass spectral drift); qualitative or quantitative, all sources, includes specimen validity testing, per day; 8-14 drug class(es), including metabolite(s) if performed
G0482	Drug test(s), definitive, utilizing (1) drug identification methods able to identify individual drugs and distinguish between structural isomers (but not necessarily stereoisomers), including, but not limited to GC/MS (any type, single or tandem) and LC/MS (any type, single or tandem and excluding immunoassays (e.g., IA, EIA, ELISA, EMIT, FPIA) and enzymatic methods (e.g., alcohol dehydrogenase)), (2) stable isotope or other universally recognized internal standards in all samples (e.g., to control for matrix effects, interferences and variations in signal strength), and (3) method or drug-specific calibration and matrix-matched quality control material (e.g., to control for instrument variations and mass spectral drift); qualitative or quantitative, all sources, includes specimen validity testing, per day; 15-21 drug class(es), including metabolite(s) if performed
G0483	Drug test(s), definitive, utilizing (1) drug identification methods able to identify individual drugs and distinguish between structural isomers (but not necessarily stereoisomers), including, but not limited to GC/MS (any type, single or tandem) and LC/MS (any type, single or tandem and excluding immunoassays (e.g., IA, EIA, ELISA, EMIT, FPIA) and enzymatic methods (e.g., alcohol dehydrogenase)), (2) stable isotope or other universally recognized internal standards in all samples (e.g., to control for matrix effects, interferences and variations in signal strength), and (3) method or drug-specific calibration and matrix-matched quality control material (e.g., to control for instrument variations and mass spectral drift); qualitative or quantitative, all sources, includes specimen validity testing, per day; 22 or more drug class(es), including metabolite(s) if performed
G0659	Drug test(s), definitive, utilizing drug identification methods able to identify individual drugs and distinguish between structural isomers (but not necessarily stereoisomers), including but not limited to GC/MS (any type, single or tandem) and LC/MS (any type, single or tandem), excluding immunoassays (e.g., IA, EIA, ELISA, EMIT, FPIA) and enzymatic methods (e.g., alcohol dehydrogenase), performed without method or drug-specific calibration, without matrix-matched quality control material, or without use of stable isotope or other universally recognized internal standard(s) for each drug, drug metabolite

CPT	Code Description
	or drug class per specimen; qualitative or quantitative, all sources, includes specimen validity testing, per day, any number of drug classes

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

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Revision History

Revision Date	Summary of Changes
03/06/2024	Reviewed and Updated: Updated the background, guidelines and recommendations, and evidence-based scientific references. Literature review did not necessitate any modifications to coverage criteria.
05/31/2023	Off-Cycle Review: Proprietary test from Newstar Medical (RiskViewRx) that was called out in CC4 was replaced by CareView360. No further updates outside of the CC. Removed CPT code 0143U – 0150U (deletion effective 7/1/2023)
03/01/2023	Reviewed and Updated: Updated the background, guidelines and recommendations, and evidence-based scientific references. Literature review did not necessitate any modifications to coverage criteria. The following edits were made for clarity: All CC edited for clarity and consistency. Removed CC3 "3) Confirmatory/definitive qualitative or quantitative drug testing DOES NOT MEET COVERAGE CRITERIA when laboratory-based definitive drug testing is requested without any prior presumptive screening test results indicating the clinical utility to confirm those results." Added CPT 0328U.

03/09/2022	Annual review: updated background, guidelines and recommendations, and evidence-based scientific references. Literature review necessitated the following changes to the coverage criteria: CC1a: deleted "or substance abuse or dependence" and "at the following frequency". CC1b: changed "as a result of" to "based on" for clarity CC1g: changed "testing of" to "In" for clarity CC1h: changed "drug testing" to "In" for clarity CC1i: changed "random urine presumptive drug testing for" to "For the" for clarity CC1i, i: added "random" for clarity CC1i, ii: added "random" for clarity, and changed "in one" to "per" for clarity CC2: added several commas and verbs for clarity. The new criteria now reads "Confirmatory/definitive qualitative or quantitative drug testing **MEETS COVERAGE CRITERIA,** up to seven drug classes, when laboratory-based definitive drug testing is specifically requested, the rationale is documented by the patient's treating physician, and **ANY** of the following conditions are met:" CC2a: added "their" for clarity CC2a, i and ii: changed commas to semicolons CC2b: changed the period at the end of the sentence to a colon CC2b, i, ii, and iii: deleted "For" and "meets coverage criteria" for clarity CC2e: Added "To" for clarity
06/02/2021	Off-cycle Review: Updated background, guidelines and recommendations, and evidence-based scientific references. Literature review did necessitate following modifications to the coverage criteria: Addition for clarity: If there is a conflict between this Policy and any relevant, applicable government policy [e.g. National Coverage Determinations (NCD) for Medicare] for a particular member, then the government policy will be used to make the determination. Addition for clarity of "up to seven drug classes" to CC2. Addition of the following CC for clarity: • Confirmatory/definitive qualitative or quantitative drug testing **DOES NOT MEET COVERAGE CRITERIA** when laboratory-based definitive drug testing is requested without any prior presumptive screening test results indicating the clinical utility to confirm those results. • Confirmatory/definitive qualitative or quantitative drug testing **DOES NOT MEET COVERAGE CRITERIA** when laboratory-based definitive drug testing is requested for larger than seven drug classes panels because evidence-based peer-reviewed literature and clinical rationale is lacking for this type of testing in the outpatient setting. • Confirmatory/definitive qualitative or quantitative or presumptive (qualitative, semi-quantitative or quantitative) drug testing using proprietary tests such as RiskViewRx Plus **DOES NOT MEET COVERAGE CRITERIA** because those tests have predetermined drug panels that are not based on the patient's unique medical history, presumptive screening results or current clinical presentation. Reworded CC1 in Reimbursement section: From: 1. Panel coding using G0480 (1 – 7 drug classes) **IS ALLOWED** for all circumstances that meet the coverage criteria indicated within "Indications and/or Limitations."

	To: 1. The following IS reimbursed (see complete Coverage Criteria in Letters A and B, Section III above) for: a. Presumptive drug screening based upon appropriate clinical criteria (qualitative, semi-quantitative or quantitative); b. Definitive drug testing (qualitative or quantitative) for up to seven drug classes when the presumptive drug screening meets one of the following criteria: i. The test was negative for prescribed medications, or ii. Positive for a prescription drug with abuse potential which was not prescribed, or iii. Positive for an illegal drug, or iv. A presumptive test does not exist or does not adequately detect the specific drug or metabolite to be tested Addition of the following CC to Reimbursement section: • Blood specimens in patients with anuric Chronic Renal Failure. Removal of the following CC from Reimbursement section: • Any panel coding other than G0480 (1-7 drug classes) • Any PLA codes for toxicology or drug testing • Confirmatory/definitive drug testing **IS ALLOWED** as meeting coverage criteria based on patient specific indications, including stage of screening, treatment, or recovery, medication response, and clinical assessment, and when accurate results are necessary to make clinical decisions. Replaced "Strictly prohibited" wording with "is not reimbursed".
03/03/2021	Annual Review: Updated background, guidelines and recommendations, and evidence-based scientific references. Literature review did not necessitate any modification to the coverage criteria. 0227U was added
09/08/2020	Off-cycle Review: Updated background, guidelines, and evidence-based scientific references. Literature review necessitated a change to coverage criteria. Based on 2020 SAMHSA guidelines, item 4 of Section IV (Reimbursement) now reads: Only urine or oral fluid specimens will be covered except blood specimen will be covered for patients with anuric Chronic Renal Failure.
03/10/2020	Annual Review: Updated background, guidelines and recommendations, and evidence-based scientific references. Literature review did not necessitate any modification to the coverage criteria.
03/01/2019	Annual Review: This policy is now being reviewed annually in quarter 1 to realign the review schedule. Updated background, guidelines, and evidence-based scientific references. Literature review did necessitate modifications to CC. • For clarity, added separate CC stating that panel coding using G0480 (1 – 7 drug classes) **MCC** for all circumstances listed in previous coverage criteria as part of reimbursement section. • For clarity, stated a subpoint that any AMA definitive drug class codes and a separate subpoint that any PLA codes for toxicology or drug testing **DNMCC** as part of reimbursement section • Added individuals with a diagnosed mental illness **MCC** for presumptive drug screening per recommendations of 2013 AACAP, 2017 AACC, 2014 ADAC, 2016 APA, and 2015 WFSBP • Added individuals with attention-deficit hyperactivity and disruptive behavior disorders **MCC** for presumptive drug screening

	• Added: Drug testing in individuals with epilepsy **MCC** per recommendations of 2018 NICE and 2018 AAN. • Added CC that specific validity testing, including genetic identity testing, **DNMCC** • Added individuals where substance abuse is in the differential diagnosis of the presenting conditions EXCEPT as part of a general encounter **MCC** for presumptive drug screening • Reordered and rearranged CCs to separate out presumptive screening from definitive testing Added PLA codes 0006U, 0007U, 0011U, 0051U, 0054U and 0082U.
06/11/2018	Annual review: Background, Literature Review, Applicable Federal Regulations, Guidelines and Recommendations and Evidence-based Scientific References were updated. Literature review did necessitate modifications to CC: language was clarified and reworded regarding opioids, presumptive and definitive drug testing. Title was changed.
06/02/2017	Annual review: Added guidelines from CDC and Palmetto GBA LCD in the Guidelines and Recommendations section.
01/1/2017	Off-cycle review: New CPT codes added: 80305, 80306 and 80307 to replace CMS HCPCS codes G0477, G0478 and G0479 respectively per AMA 2017 updates
12/1/2016	Off-cycle review: Revised coverage criteria 1 to remove reference to testing in the ER situation, as outside of Avalon scope of services.
07/26/2016	Annual review: added coverage criteria 2, 3, 4 and 6A. Added content on random testing, Reimbursement Limitation 1 and policy exclusions.
02/26/2016	Off-cycle review: revision to coverage criteria to reflect removal of hair as specimen type
11/16/2015	Off-cycle review: Coverage criteria revision to reflect CMS Palmetto LCD released 4Q15
06/06/2015	Initial presentation

Prostate Biopsy Specimen Analysis

Policy Number: AHS – G2007 – Prostate Biopsy Specimen Analysis	Prior Policy Name and Number, as applicable: AHS – G2007 – Prostate Biopsies
Initial Presentation Date: 09/18/2015 Last Review Date: February 1, 2025	

Policy Description

Prostate cancer is characterized by a malignancy of the small walnut-shaped gland that produces seminal fluid. This malignancy can present with a wide clinical range, from only being a microscopic, well-differentiated tumor that may never be clinically significant all the way to being an aggressive, high-grade cancer (Taplin & Smith, 2023).

Related Policies

Policy Number	Policy Title
AHS-G2008	Prostate Specific Antigen (PSA) Testing
AHS-G2013	Testosterone
AHS-G2054	Liquid Biopsy
AHS-G2124	Serum Tumor Markers for Malignancies
AHS-M2066	Genetic Cancer Susceptibility Using Next Generation Sequencing
AHS-M2166	Gene Expression Profiling and Protein Biomarkers for Prostate Cancer

Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) In the initial diagnosis of prostate cancer as a follow up to abnormal PSA results, presence of a palpable nodule on digital rectal examination, **or** suspicious radiologic findings, pathological examination of tissue obtained from a prostate biopsy involving 12 core extended sampling (see Note 1 below) **MEETS COVERAGE CRITERIA**.

2) When the clinical suspicion of prostate cancer remains in an individual for whom an initial biopsy was negative for prostate cancer, pathological examination of tissue from a follow-up prostate biopsy (excluding prostate saturation biopsy) **MEETS COVERAGE CRITERIA**.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

3) Pathological examination of tissue obtained from a prostate saturation biopsy **DOES NOT MEET COVERAGE CRITERIA** for the diagnosis, staging, or management of prostate cancer.

NOTES:

Note 1: One vial per sextant, with no more than two core samples per vial.

Table of Terminology

Term	Definition
ACR	American College of Radiology
ACS	American Cancer Society
ASCO	American Society of Clinical Oncology
ASTRO	American Society for Radiation Oncology
AUA	American Urological Association
CC	Cubic centimeters
CLIA '88	Clinical Laboratory Improvement Amendments Of 1988
CMS	Centers For Medicare and Medicaid
CS	Clinically significant
csPCa	All clinically significant cases of prostate cancer
DRE	Digital rectal examination
EAU	European Association of Urology
ESMO	European Society for Medical Oncology
FBx	Fusion biopsy
FDA	Food And Drug Administration
GG2	Grade 2 or greater
LDT	Laboratory-developed test
mpMRI	Multi-parametric magnetic resonance imaging
MRI	Magnetic resonance imaging

NCCN	National Comprehensive Cancer Network
NPV	Negative predictive value
NYU	New York University
PI-RADS	Prostate imaging reporting and data system
PPV	Positive predictive value
PROMIS	Prostate magnetic resonance imaging study
PSA	Prostate specific antigen
RP	Radical prostatectomy
SBx	TRUS biopsy
SUO	Society Of Urologic Oncology
TPM	Template prostate mapping
TRUS	Transrectal ultrasound
UCLA	University Of California, Los Angeles
US	Ultrasound
USPSTF	United States Preventive Services Task Force

Scientific Background

Prostate cancer is the most common cancer in American individuals with a prostate and the second leading cause of death in individuals with a prostate who are 65 years of age or older (Balducci et al., 1997; Tabayoyong & Abouassaly, 2015) with an estimated 288,300 new cases and 34,700 deaths in the US in 2023 (Siegel et al., 2023). About 11% of individuals with a prostate will be diagnosed with prostate cancer during their lifetime (Taplin & Smith, 2023).

Many cases of prostate cancer do not become clinically evident, as indicated in autopsy series of individuals with a prostate- prostate cancer is detected in approximately 30% of these individuals at age 55 and approximately 60% of these individuals by age 80 (Bell et al., 2015). These data suggest that prostate cancer often grows so slowly that most affected individuals die of other causes before the disease becomes clinically advanced. Prostate cancer survival is related to many factors, especially the extent of tumor at the time of diagnosis. The five-year relative survival among individuals with cancer confined to the prostate (localized) or with just regional spread is 100%, compared with 31% among those diagnosed with distant metastases (Hoffman & Preston, 2023).

Findings on digital rectal examination (DRE) including the presence of nodules, induration, or asymmetry or elevated prostate specific antigen (PSA) levels indicate the need for prostate biopsy. Although considered safe, prostate biopsy is an invasive procedure and recommendations for its use are limited to a subset of patients. Screening the general population for prostate cancer remains a controversial issue (Hoffman & Preston, 2023). Screening may reduce the risk of distant-stage prostate cancer. The European Randomized Study of Screening for Prostate Cancer (ERSPC) enrolled 162,243 individuals with a prostate ages 50 to 69 years. The cumulative incidence rate of metastatic disease in the regular screening group was 0.67 percent compared to the control group of 0.86 percent. The absolute risk reduction of metastatic disease was 3.1 per 1000 individuals randomized (Hoffman & Preston, 2023) .

Multiple sampling schemes have been developed to improve the accuracy of prostate biopsy in the detection of cancer. Systematic prostate sampling is performed and augmented by additional sampling of any abnormal areas found on ultrasound or rectal examination (Gosselaar et al., 2008). During transrectal ultrasound (TRUS)-guided biopsy, a six-core, or sextant biopsy technique, takes one sample each from the apex, base, and mid-prostate on each side (Hodge et al., 1989). However, this method

may miss approximately 30% of clinically significant cancers and has been replaced by extended core biopsy which obtains five to seven evenly-distributed specimens from each side, sampling more extensively from the lateral aspects of the prostate (Benway & Andriole, 2021). A meta-analysis by Eichler et al. (2006) found that schemes with 12 core samples that took additional laterally directed cores detected 31% more cancers compared with a six-core approach, with increasing number of cores significantly associated with increased detection of prostate cancer (Eichler et al., 2006). This biopsy method has been used to obtain up to 18 cores for evaluation (Benway & Andriole, 2021).

Saturation biopsy involves extensive sampling of the prostate, obtaining up to 24 core samples. Saturation biopsy is not appropriate for initial screening as it does not provide increased cancer detection when used for first-time biopsy but may provide increased sensitivity when repeat biopsies are performed and can be considered after one or more negative TRUS-biopsies. Saturation biopsy detects prostate cancer in approximately 22% to 33% of patients undergoing repeat biopsy, but it is associated with a higher incidence of complications (Benway & Andriole, 2021).

Complications may occur with biopsy. Firstly, the samples from a biopsy may be inadequate to make a diagnosis; the cores obtained may not be of high enough quality or more cores may be needed. Other findings such as an abnormal but nonmalignant histology may warrant a repeat biopsy. Clinical complications such as inflammation, bleeding, infection, and urinary obstruction are also possible (Benway & Andriole, 2021). Pepe and Aragona (2007) estimated the rate of clinical complication after a transperineal biopsy to be as high as 40% (Pepe & Aragona, 2007).

Clinical Utility and Validity

Thompson et al. (2015) studied whether saturation or transperineal biopsy altered oncological outcomes as compared with standard transrectal biopsy. In total, 650 individuals with a prostate were analyzed, and saturation biopsy was associated with "increased objective biopsy progression requiring treatment" on both the Kaplan-Meier analysis and multivariate Cox analysis. A logistic regression analysis of 179 individuals undergoing a radical prostatectomy (RP) found that transperineal biopsy was associated with lower likelihood of "unfavourable" RP pathology. The authors concluded that "saturation biopsy increased progression to treatment on AS; longer follow-up is needed to determine if this represents beneficial earlier detection of significant disease or over-treatment. Transperineal biopsy reduced the likelihood of unfavourable disease at RP, possibly due to earlier detection of anterior tumours" (Thompson et al., 2015).

Zaytoun et al. (2011) "compared saturation and extended repeat biopsy protocols after initially negative biopsy." The study included 1056 individuals with a prostate- 393 of these individuals underwent a 1214 core biopsy ("extended") and 663 of these individuals underwent a 20-24 core biopsy ("saturated"). Overall, prostate cancer was detected in 315 patients, but saturated biopsy detected a third more cancers and identified more cancers in a benign initial biopsy. In total, 119 biopsies identified clinically "insignificant" cancer. The authors concluded, "Compared to extended biopsy, office-based saturation biopsy significantly increases cancer detection on repeat biopsy. The potential for increased detection of clinically insignificant cancer should be weighed against missing significant cases" (Zaytoun et al., 2011).

The Prostate Magnetic Resonance Imaging Study (PROMIS) study (Brown et al., 2018) assessed the ability of multi-parametric MRI (mpMRI) to identify individuals with a prostate who could safely avoid an "unnecessary biopsy" and compared mpMRI to TRUS-guided biopsy. A TPM-biopsy was included for comparison, and 576 individuals with a prostate underwent all three tests. Clinically significant (CS) cancer was defined as "a Gleason score of ≥ 4 + 3 and/or cancer core length of ≥ 6 mm." For CS cancer,

TRUS-guided biopsy showed a sensitivity of 48%, specificity of 96%, PPV of 90%, and NPV of 74%. The sensitivity of mpMRI was 93%, specificity was 41%, PPV was 51%, and NPV was 89%. A negative mpMRI scan was recorded for 158 individuals with a prostate (27%). Of these, 17 were found to have CS cancer on TPM-biopsy. The authors also found that the most cost-effective strategy involved testing all individuals with a prostate with "mpMRI, followed by MRI-guided TRUS-guided biopsy in those patients with suspected CS cancer, followed by rebiopsy if CS cancer was not detected" (Brown et al., 2018).

Sidana et al. (2018) compared the yield of MRI fusion biopsy (FBx) to 12-core TRUS biopsy (SBx) in patients with prior negative biopsies. The study included 779 patients, and a total of 346 cancers were detected with 239 of 346 considered clinically significant. FBx diagnosed a total of 205 patients with SBx diagnosing an additional 34 patients. FBx identified high proportions of clinically significant cancers over all amounts of prior negative biopsies. The authors stated that "SBx added a relatively small diagnostic value to FBx for detecting CS disease" and concluded that "repeat SBx alone in patients with multiple prior negative biopsies will be hindered by lower yield and FBx should be utilized concurrently in these patients" (Sidana et al., 2018).

Pepe et al. (2018) investigated the diagnostic accuracies for clinically significant prostate cancer, multiparametric magnetic resonance imaging (MRI) and transperineal saturation prostate biopsy. Lesions with PI-RADS (Prostate Imaging Reporting and Data System) scores of three or higher were subjected to additional targeted fusion prostate biopsy. A total of 1032 patients were included, with 372 deemed to have T1c prostate cancer. Further, 272 of these cases were considered "clinically significant." Saturation biopsy missed 12 of 272 clinically significant cancers, and targeted fusion prostate biopsy with the score cutoff of three missed 44 cases. However, the authors noted that using multiparametric MRI in combination with a score cutoff of 3 in PI-RADS would have prevented 49.3% of biopsies, and a score cut-off of 4 would have prevented 73.6% of biopsies, although the score cutoff of 4 missed 108 of 272 clinically significant cases. The authors concluded that multiparametric MRI could "significantly reduce the number of unnecessary repeat prostate biopsies in about 50% of cases in which a PI-RADS score of 3 or greater is used" (Pepe et al., 2018).

Pepe et al. (2020) investigated the number of cores (combined with multiparametric MRI [mpMRI]) needed to diagnose all clinically significant cases of prostate cancer (csPCa) in individuals with a prostate who were subject to transperineal saturation biopsy (SPBx; 30 cores). The study included 875 patients and stage 1 prostate cancer was found in 306 of these patients, with 222 of these classified as clinically significant. The initial 20 needle cores obtained from SPBx identified all 222 cases of clinically significant prostate cancer, although it missed 84 of 129 indolent cases. Overall, the "diagnostic accuracy, sensitivity, and specificity [were] equal to 83.1%, 100%, and 65.1%, respectively." The authors concluded that in individuals with a prostate who were "subject to mpMRI and/or TPBx, a maximum of 20 systematic transperineal needle cores detected all cases of csPCa and minimized the diagnosis of indolent cancers" (Pepe et al., 2020).

Klotz et al. (2021) investigated magnetic resonance imaging (MRI) with targeted biopsy against TRUS-guided biopsy to determine whether MRI with a targeted biopsy was as effective in detecting a grade 2 or greater prostate cancer. In total, 453 individuals underwent tests and were randomized to receive TRUS biopsy or MRI-TB. Cancers of grade 2 or greater (GG2) were identified in 67 of 225 individuals (30%) who underwent TRUS biopsy vs 79 of 227 (35%) allocated to MRI-TB. The authors concluded that "magnetic resonance imaging followed by selected targeted biopsy is noninferior to initial systemic biopsy in [individuals] at risk for prostate cancer in detecting GG2 or greater cancers" (Klotz et al., 2021).

Lokeshwar et al. (2022) studied the clinical utility of mpMRI guided prostate biopsy. The study started with a retrospective analysis of 415 individuals with low-risk prostate cancer that was being managed with active surveillance. Then, 125 participants were selected based on having a mpMRI visible index lesions score of 2 or 3 according to PI-RADS version 2. Clinically significant prostate cancer, defined as Gleason grade group of at least 2, was found in 22 of 125 patients (17.6%). The authors found that the only significant variable that could predict detection was "higher PSAD." The authors conclude that "integration of PSAD may be a useful adjunctive tool in identifying patients at highest risk for upgrade despite favorable imaging findings" (Lokeshwar et al., 2022).

Guidelines and Recommendations

The American Urological Association (AUA)

The AUA published a paper (2015) on Optimal Techniques of Prostate Biopsy and Specimen Handling which recommended: "12-core systematic sampling methodology that incorporates apical and far-lateral cores in the template distribution. The results of our literature review suggest that collecting more than 12 cores or sampling the transition zone offer no benefit for initial diagnostic biopsies. However, such approaches might be useful for resampling following a negative biopsy" (Samir et al., 2015).

The AUA/American Society for Radiation Oncology (ASTRO)/Society of Urologic Oncology (SUO) published guidelines (Sanda et al., 2018) which state:

- "Localized prostate cancer patients who elect active surveillance should have accurate disease staging including systematic biopsy with ultrasound or MRI-guided imaging."
- "Localized prostate cancer patients undergoing active surveillance should be encouraged to have a confirmatory biopsy within the initial two years and surveillance biopsies thereafter."

In 2018, the American Society of Clinical Oncology (ASCO) endorsed the above 2017 AUA/ASTRO/SUO joint guideline, with only a minor disagreement on two cryosurgery recommendations (Bekelman et al., 2018).

In 2020, The American Urological Association and the Society of Abdominal Radiology Prostate Disease Focus Panel published a guideline (Bjurlin et al., 2020) on standard operating procedures for multiparametric magnetic resonance imaging in the diagnosis, staging, and management of prostate cancer. The guideline states:

- "mpMRI of the prostate allows for risk stratification of [individuals] at risk for prostate cancer including its ability to predict cancer aggressiveness prior to biopsy."
- "The performance of prostate mpMRI in [individuals] with no prior biopsy is now supported by randomized clinical trials, while its use in [individuals] with a prior negative biopsy continues to be endorsed by consensus statements and national guidelines" (Bjurlin et al., 2020).

In 2023, the AUA and SUO released guidelines on early detection of prostate cancer (Wei et al., 2023). They recommend the following regarding prostate biopsies.

In terms of PSA screening:

- "For people with a newly elevated PSA, clinicians should repeat the PSA prior to a secondary biomarker, imaging, or biopsy."

- "For people undergoing prostate cancer screening, clinicians should not use PSA velocity as the sole indication for a secondary biomarker, imaging, or biopsy."
- "Clinicians and patients may use validated risk calculators to inform the SDM process regarding prostate biopsy."
- "When the risk of clinically significant prostate cancer is sufficiently low based on available clinical, laboratory, and imaging data, clinicians and patients may forgo near-term prostate biopsy."

In terms of initial biopsy:

- "Clinicians should inform patients undergoing a prostate biopsy that there is a risk of identifying a cancer with a sufficiently low risk of mortality that could safely be monitored with active surveillance (AS) rather than treated."
- "Clinicians may use magnetic resonance imaging (MRI) prior to initial biopsy to increase the detection of Grade Group (GG) 2+ prostate cancer."
- "For biopsy-naïve patients who have a suspicious lesion on MRI, clinicians should perform targeted biopsies of the suspicious lesion and may also perform a systematic template biopsy. "
- "For patients with both an absence of suspicious findings on MRI and an elevated risk for GG2+ prostate cancer, clinicians should proceed with a systematic biopsy."
- "Clinicians may use adjunctive urine or serum markers when further risk stratification would influence the decision regarding whether to proceed with biopsy."
- "For patients with a PSA > 50 ng/mL and no clinical concerns for infection or other cause for increased PSA (e.g., recent prostate instrumentation), clinicians may omit a prostate biopsy in cases where biopsy poses significant risk or where the need for prostate cancer treatment is urgent (e.g., impending spinal cord compression)."

In terms of repeat biopsy:

- Clinicians should communicate with patients following biopsy to review biopsy results, reassess risk of undetected or future development of GG2+ disease, and mutually decide whether to discontinue screening, continue screening, or perform adjunctive testing for early reassessment of risk."
- "Clinicians should not discontinue prostate cancer screening based solely on a negative prostate biopsy."
- "After a negative biopsy, clinicians should not solely use a PSA threshold to decide whether to repeat the biopsy."
- "If the clinician and patient decide to continue screening after a negative biopsy, clinicians should re-evaluate the patient within the normal screening interval (two to four years) or sooner, depending on risk of clinically significant prostate cancer and life expectancy."
- "At the time of re-evaluation after negative biopsy, clinicians should use a risk assessment tool that incorporates the protective effect of prior negative biopsy."
- "After a negative initial biopsy in patients with low probability for harboring GG2+ prostate cancer, clinicians should not reflexively perform biomarker testing."
- "After a negative biopsy, clinicians may use blood, urine, or tissue-based biomarkers selectively for further risk stratification if results are likely to influence the decision regarding repeat biopsy or otherwise substantively change the patient's management."

- "In patients with focal (one core) high-grade prostatic intraepithelial neoplasia (HGPIN) on biopsy, clinicians should not perform immediate repeat biopsy."
- "In patients undergoing repeat biopsy with no prior prostate MRI, clinicians should obtain a prostate MRI prior to biopsy."
- "In patients with indications for a repeat biopsy who do not have a suspicious lesion on MRI, clinicians may proceed with a systematic biopsy."
- "In patients undergoing repeat biopsy and who have a suspicious lesion on MRI, clinicians should perform targeted biopsies of the suspicious lesion and may also perform a systematic template biopsy."

In terms of biopsy technique:

- "Clinicians may use software registration of MRI and ultrasound images during fusion biopsy, when available."
- "Clinicians should obtain at least two needle biopsy cores per target in patients with suspicious prostate lesion(s) on MRI."
- "Clinicians may use either a transrectal or transperineal biopsy route when performing a biopsy."

National Comprehensive Cancer Network (NCCN)

The NCCN Guidelines on Early Detection for Prostate Cancer state that "systematic prostate biopsy under TRUS guidance with or without targeted of lesions seen on pre-biopsy MRI is the recommended technique for prostate biopsy." It recommends the use of an extended pattern at least 12 core biopsies as it has been validated and results in enhances cancer detection compared to sextant biopsy schemes. Moreover, the NCCN states,

- "Anteriorly directed biopsy is not supported (NCCN, 2023a, 2023b) in routine biopsy. However, this can be added to an extended biopsy protocol in a repeat biopsy if PSA is persistently elevated."
- "A negative biopsy does not preclude a diagnosis of prostate cancer on subsequent biopsy. If clinical suspicion of cancer persists after a negative biopsy, consideration can be given to saturation biopsy strategies and/or the use of multiparametric MRI followed by an appropriate targeted biopsy technique based on the results."
- Despite this emerging evidence, the panel does not recommend a saturation biopsy strategy for all individuals with a prostate with "previous negative biopsies at this time given the benefits seen for MRI and MRI-targeted biopsy in this patient population."
- "After one or more negative TRUS biopsies, [individuals] who are considered high-risk (e.g. those with persistently elevated or rising PSA) can be considered for MRI followed by targeted biopsy." The NCCN notes that targeted biopsy techniques include "cognitive or visual targeting, TRUS-MRI fusion platforms, and direct in-bore magnetic resonance biopsy-guided biopsy.
- "Overall, the panel believes that the data for the use of MRI and MRI-targeted biopsies in the initial biopsy setting are increasingly compelling. However, studies using both targeted and systematic sampling routinely demonstrate higher yield of clinically significant cancer with the combined approach and improved sensitivity. Therefore, a combination of systematic and targeted procedures is preferred when MRI-targeting capabilities are available, at least at initial biopsy."

The NCCN also addressed prostate biopsy in their Prostate Cancer guideline. The NCCN remarks that biopsy (and/or multiparametric MRI) can be considered for active surveillance for patients with over 10 years life expectancy. The NCCN also states that a prostate biopsy should not be repeated "no more often than 12 months" unless clinically indicated (such as PSA increase) (NCCN,2022a).

American College of Radiology (ACR)

The ACR (Coakley et al., 2017) rated TRUS guided biopsy a 9, and MRI targeted prostate biopsy a 7 in the most recent ACR Appropriateness Criteria for Prostate Cancer Pretreatment Detection, Surveillance and Staging for "clinically suspected prostate cancer with no prior biopsy." A rating of 7, 8 or 9 are usually appropriate. MRI targeted biopsy was rated an 8 and repeat TRUS biopsy rated a 7 in "clinically suspected prostate cancer, prior negative TRUS biopsy" as well as "clinically established low risk prostate cancer for active surveillance."

They note that "Overall, the clinical paradigm for prostate cancer diagnosis is rapidly moving towards MRI-targeted transrectal biopsy, based on substantial evidence from several centers (notably the National Institutes of Health; New York University [NYU]; University of California, Los Angeles [UCLA]; and Nijmegen) that this approach can transform baseline cancer evaluation when compared with traditional systematic biopsy, with fewer false negatives, better tumor characterization, improved tumor localization, and better treatment stratification, especially stratification to lower-risk cohorts that may be appropriate for active surveillance or focal therapy" (Coakley et al., 2017).

American Cancer Society (ACS)

The ACS published guidelines (Wolf et al., 2010) which state:

> "A PSA level of 4.0 ng/mL or greater historically has been used to recommend referral for further evaluation or biopsy, which remains a reasonable approach for [individuals] at average risk for prostate cancer."
> "For PSA levels between 2.5 ng/mL and 4.0 ng/mL, health care providers should consider an individualized risk assessment that incorporates other risk factors for prostate cancer, particularly for high-grade cancer, that may be used to recommend a biopsy. Factors that increase the risk of prostate cancer include African American race, family history of prostate cancer, increasing age, and abnormal DRE. A previous negative biopsy lowers the risk. Methods are available that merge this information to achieve an estimate of a man's overall risk of prostate cancer and, more specifically, of his risk of high-grade prostate cancer."

According to the ACS, an update to the guidelines for prostate cancer was initiated in 2019 (Smith et al., 2019).

United States Preventive Services Task Force

Within the 2018 USPSTF recommendation statement regarding prostate screening, they state that for individuals with a prostate "with a positive PSA test result may undergo a transrectal ultrasound-guided core-needle biopsy of the prostate to diagnose prostate cancer… Although protocols vary, active surveillance usually includes regular, repeated PSA testing and often repeated digital rectal examination and prostate biopsy, with potential for exposure to repeated harms from biopsies" (USPSTF, 2018).

European Society for Medical Oncology (ESMO)

The ESMO includes recommendations for prostate biopsies:

- "Transperineal biopsies are recommended, rather than transrectal ultrasound (TRUS)-guided biopsies." ESMO further noted that "Targeted transperineal biopsies, in comparison with systematic transrectal biopsies, result in an increased detection rate of clinically significant prostate cancer, a decreased detection rate of clinically insignificant prostate cancer, and fewer adverse events."
- When multiparametric MRI is positive (defined as [PI-RADS] ≥3), ESMO recommends performing a targeted (systematic or non-systematic) biopsy. However, when multiparametric MRI is negative (PI-RADS ≤2) and clinical suspicion of cancer is low, the biopsy can be omitted (Parker et al., 2020).

European Association of Urology

The EAU's recommendations on prostate biopsy include the following:

- For biopsy-naïve patients and patients with prior negative biopsy, "perform MRI before prostate biopsy."
- "Base follow-up during active surveillance (AS) on a strict protocol including digital rectal examination (at least once yearly), prostate-specific antigen (PSA) (at least once every 6 months) and repeated biopsy every 2 to 3 years."
- "Perform magnetic resonance imaging (MRI) and repeat biopsy if PSA is rising (PSA- doubling time < 3 years)."
- For asymptomatic individuals with a prostate with a "PSA level between 3–10 ng/mL and a normal DRE, use one of the following tools for biopsy indication: risk-calculator, provided it is correctly calibrated to the population prevalence; [OR] magnetic resonance imaging of the prostate" (EAU, 2023).

Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

The FDA has cleared numerous devices including needles, reagents, instrumentation, and imaging systems for use in prostate biopsy. Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
88305	Level IV – Surgical pathology, gross and microscopic examination
G0416	Surgical pathology, gross and microscopic examinations, for prostate needle biopsy, any method

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

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Revision History

Revision Date	Summary of Changes
09/06/2023	Reviewed and Updated: Updated the background, guidelines and recommendations, and evidence-based scientific references. Literature review did not necessitate any modifications to coverage criteria. The following edits were made for clarity: Title changed to "Prostate Biopsy Specimen Analysis" Addition of "pathological examination of tissue obtained from a" before "prostate biopsy" in CC1, 2, and 3, as this policy focuses on the laboratory test performed, not the surgical procedure to get the sample. Removed asterisk before "Note 1" in CC1.

Prostate Specific Antigen (PSA) Testing

Policy Number: AHS – G2008 – Prostate Specific Antigen (PSA) Testing	Prior Policy Name and Number, as applicable: G2008 – Prostate Cancer Screening
Initial Presentation Date: 06/16/2015 Revision date: 03/06/2024	

Policy Description

Prostate-specific antigen (PSA) is a glycoprotein that is produced by both normal and neoplastic prostate tissue. In normal conditions, PSA is produced as a proenzyme in the prostate and secreted into the lumen. The propeptide is removed to activate the proenzyme; from there, it undergoes proteolysis to inactivate it. This inactive form may enter the bloodstream and circulate as "free" PSA. This process differs in prostate cancer; the basal cells that normally regulate this activation process are missing, which allows the secreted PSA direct access into the bloodstream. This increases the PSA concentration in the serum (Freedland, 2024).

Due to these reasons, PSA is often used in assessment of prostate cancer, such as screening, monitoring, diagnosis, and treatment management.

Terms such as male and female are used when necessary to refer to sex assigned at birth.

Related Policies

Policy Number	Policy Title
AHS-G2007	Prostate Biopsy Specimen Analysis
AHS-M2166	Gene Expression Profiling and Protein Biomarkers for Prostate Cancer Management

Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) For average-risk individuals 45-75 years of age, screening for prostate cancer with the total prostate-specific antigen (PSA) test **MEETS COVERAGE CRITERIA**.

2) For individuals 40-75 years of age, annual screening for prostate cancer with the total PSA test **MEETS COVERAGE CRITERIA** when one of the following conditions is met:
   a) Individual is of African ancestry.
   b) Individual has germline mutations that increase risk for prostate cancer.
   c) Individual has a suspicious family history.

3) For individuals over 75 years of age who have little or no comorbidities (see Note 1), screening for prostate cancer with a total PSA test **MEETS COVERAGE CRITERIA**.

4) For individuals with previous total PSA results, repeat screening for prostate cancer with a total PSA test **MEETS COVERAGE CRITERIA** with the following frequency:
   a) For individuals less than 76 years of age, when total PSA is <1 ng/ml and digital rectal exam (DRE) is normal (if done): Repeat screening at 2- to 4-year intervals.
   b) For individuals less than 76 years of age, when total PSA is 1-3 ng/ml and DRE is normal (if done): Repeat screening at 1- to 2-year intervals.
   c) For individuals greater than 75 years of age, when total PSA is <4 ng/ml and DRE is normal (if done) and no other indications for biopsy: Repeat screening in select patients (very healthy individuals with little or no comorbidity) at 1- to 4- year intervals.

5) A percent free PSA **or** a follow-up in 6-12 months with total PSA **MEETS COVERAGE CRITERIA** when **any** of the following conditions are met:
   a) For individuals less than 76 years of age with a total PSA >3 ng/ml and/or a very suspicious DRE.
   b) For select individuals greater than 75 years of age (very healthy individuals with little or no comorbidity) with a total PSA $\geq$4 ng/ml or a very suspicious DRE.

6) For individuals thought to be at a higher risk despite at least one prior negative prostate biopsy, follow-up testing with percent free PSA **MEETS COVERAGE CRITERIA**.

7) Total PSA testing **MEETS COVERAGE CRITERIA** in **any** of the following situations:
   a) For initial prostate cancer diagnosis in individuals with signs and symptoms of prostate cancer (see Note 2).
   b) For follow-up of individuals with a current or previous diagnosis of prostate cancer.
   c) For ongoing monitoring of individuals who have undergone tumor resection or prostatectomy.
   d) For monitoring response to prostate cancer therapy.
   e) For detecting disease recurrence.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

8) The following testing **DOES NOT MEET COVERAGE CRITERIA**:
   a) Percent free PSA as a first-line screening test for prostate cancer.
   b) Percent free PSA, free-to-total PSA ratio, and/or complexed PSA tests for the routine screening of prostate cancer.

NOTES:

Note 1: According to the NCCN guidelines, "Testing after 75 years of age should be done only in very healthy men with little or no comorbidity (especially if they have never undergone PSA testing or have a rising PSA) to detect the small number of aggressive cancers that pose a significant risk if left undetected until signs or symptoms develop. Widespread screening in this population would substantially increase rates of overdetection and is not recommended" (NCCN, 2023b). Additionally, the term individuals in this policy apply to individuals who have a prostate or were born with a prostate.

Note 2: According to ACS, 2019: "Most prostate cancers are found early, through screening. Early prostate cancer usually causes no symptoms. More advanced prostate cancers can sometimes cause symptoms, such as:

- Problems urinating, including a slow or weak urinary stream or the need to urinate more often, especially at night
- Blood in the urine or semen
- Trouble getting an erection (erectile dysfunction or ED)
- Pain in the hips, back (spine), chest (ribs), or other areas from cancer that has spread to bones
- Weakness or numbness in the legs or feet, or even loss of bladder or bowel control from cancer pressing on the spinal cord" (ACS, 2023b).

Table of Terminology

Term	Definition
AACU	American Association of Clinical Urologists Inc.
AAFP	The American Association of Family Physicians
ACP	The American College of Physicians
ACS	The American Cancer Society
ADT	Androgen deprivation therapy
AMACR	Alpha-methylacyl coenzyme a racemase
AS	Active surveillance
AUA	American Urological Association
BPH	Benign prostatic hyperplasia
BRCA	*Breast cancer gene, 1/2 mutation* (refers to *breast cancer gene 1*and *breast cancer gene 2*)
CCO	Cancer Care Ontario
CDC	Centers for Disease Control and Prevention

CMS	Centers for Medicare and Medicaid Services
CTFPHC	The Canadian Task Force on Preventive Healthcare
CUA	Canadian Urological Association
DRE	Digital rectal examination
EANM	European Association of Nuclear Medicine
EAU	European Association of Urology
ED	Erectile dysfunction
ESTRO	European Society for Radiotherapy and Oncology
ESUR	European Society of Urogenital Radiology
IVDMIA	In vitro diagnostic multivariate index assay
LDTs	Laboratory-developed tests
LUGPA	The Large Urology Group Practice Association
NCCN	National Comprehensive Cancer Network
NCI	National Cancer Institute
nmCRPC	Non-metastatic castration-resistant prostate cancer
NSAIDs	Non-steroidal anti-inflammatory drugs
PCA3	*Prostate cancer gene 3*
PHI	Prostate health index
PSA	Prostate-specific antigen
SIOG	International Society of Geriatric Oncology
SUO	Society of Urologic Oncology
TRUS	Transrectal ultrasound
USPSTF	The United States Preventive Services Task Force

Scientific Background

Prostate cancer is the most common cancer in American men and the second leading cause of death in men over 65 (Balducci et al., 1997; Tabayoyong & Abouassaly, 2015). According to the CDC (2022a), more than 224,000 prostate cancer cases are reported annually in the United States, leading to more than 31,000 prostate cancer deaths each year. The American Cancer Society estimates over 288,000 new cases and 34,000 deaths of prostate cancer in 2023 (American Cancer Society, 2023). Prostate cancer survival is related to many factors, especially the extent of the tumor at the time of diagnosis. The 5-year survival rate for men with localized or regional prostate cancer is nearly 100%, while the 5-year survival rate for men with distant prostate cancer, where the cancer has spread to other parts of the body such as the lungs, liver or bones, is 31% (ACS, 2024; Preston, 2023). About one man in nine will be diagnosed with prostate cancer during his lifetime in the United States (American Cancer Society, 2023).

Many cases of prostate cancer do not become clinically evident, as indicated in autopsy studies, where prostate cancer is detected in approximately 30 percent of men aged 55 or older and approximately 60 percent of men by age 80 (Bell et al., 2015). These data suggest that prostate cancer often grows so slowly that most men die of other causes before the disease becomes clinically advanced (Preston, 2023).

Most prostate cancers use androgen-dependent signaling for development and progression (Fisher et al., 2015). As the number of targeted therapy agents increase, it is crucial to determine which patients

will benefit from these interventions. Understanding the molecular pathology will allow clinicians to provide better patient management. Recent studies have led to the classification of prostate cancer into different subtypes, yet the utility of this in the clinical setting is to be determined (Rodrigues et al., 2014).

Prostate-specific antigen (PSA), a glycoprotein produced by prostate epithelial cells, is the most widely accepted biomarker for prostate cancer screening. Levels of this protein can be identified via a simple blood test; many doctors consider abnormal PSA levels to be above 4.0 ng/mL, although there is no official standardized normal or abnormal PSA level (NCI, 2022). Further, PSA levels tend to increase with age, suggesting that age-specific PSA reference ranges may be important for clinical use (NCI, 2022).

In serum, PSA can be identified in three forms. The main form is PSA bound by alpha-1 antichymotrypsin and accounts for approximately 75% of total PSA; PSA bound to alpha-2 macroglobulin has also been identified but cannot be detected by commercial immunoassays and represents less than 0.1% of PSA (Prcic et al., 2016). Finally, unbound or free PSA, which is the enzymatically inactive form, can be found in 5-50% of serum samples (Prcic et al., 2016). Total PSA measures the amount of all PSA identified in a sample. Some researchers claim that the amount of total versus free PSA in a sample can foreshadow prostate cancer risk (Prcic et al., 2016). Coban et al. (2016) reported that, while total PSA levels are an important prognostic factor for predicting prostate volumes, free PSA levels had a higher predictive value.

Analytical Validity

Prostate-specific antigen (PSA) was originally introduced as a tumor marker to detect cancer recurrence or disease progression following treatment (Brawley et al., 2018). It has been widely adopted for early detection of prostate cancer screening; however, its clinical utility in screening is controversial, and guidelines for PSA screening are conflicting. Non-optimal screening and treatment practices, including excessive screening among older men with lower life expectancy or comorbidities and overtreatment of men with low-risk tumors, have contributed to treatment-related harm and a lower quality of life (Fleshner et al., 2017). Evidence is currently lacking to show that PSA screening actually saves lives; instead, it may only cause overdiagnosis and lead to complications in the treatment of indolent diseases (Ilic et al., 2018).

As PSA is not a cancer-specific marker, it causes many false results that conflict with other screening methods, such as the digital rectal examination (DRE) (Saini, 2016). For example, PSA may be elevated due to conditions including benign prostatic hyperplasia (BPH) or prostatitis. This is particularly important as BPH is common among men over 50, the most common age group in which prostate cancer is observed. A study performed by Stimac et al. (2014) found PSA levels to be unusual despite testing negative for cancer. The authors concluded that subclinical inflammation had a major influence on free PSA levels only if the total levels were <10 ng/mL, and further note that clinical and acute inflammation produce a different profile of PSA release compared to a subclinical inflammation. Overall, the authors state that the molecular cause of the inflammation's changes to PSA forms are still unknown (Stimac et al., 2014). Furthermore, serum PSA is directly tied to the size of the prostate, which increases with age. Older men may see an increased concentration of PSA despite being completely healthy (Freedland, 2024; Stimac et al., 2014). Other factors such as medication can also affect PSA levels. Common medications, including statins, NSAIDs, acetaminophen, 5-alpha-reductase inhibitors, and thiazides, were all found to reduce PSA levels by varying degrees (Chang et al., 2010; Hamilton et al., 2008; Singer et al., 2008; Wang et al., 1997).

Clinical Validity and Utility

The utility of PSA-based screening is also in question. A randomized clinical trial focusing on men undergoing a single PSA-based screening (n = 189386) compared to controls not undergoing a PSA-based screening (n = 219439) found no difference in cancer mortality after a median follow-up of 10 years. The mortality rate in 1,000 individuals was 0.30 in the intervention group compared to 0.31 in the control group, or one extra death per 100,000 patients. Although prostate cancer was diagnosed more often in the intervention group (4.3% compared to 3.6% in the control group), the mortality rate was almost identical between both groups (Martin et al., 2018).

A systematic review and meta-analysis of 341,342 patients evaluated the overall effectiveness of prostate cancer screening. Results from this study showed that while PSA screening did lead to an increase in the identification of prostate cancer cases at all stages, it did not necessarily reduce the amount of overall or disease specific mortality rates (Ilic et al., 2018). This highlights the uncertainty regarding the effectiveness of prostate cancer screening. The authors also noted that "PSA screening is associated with considerable biopsy-related and cancer treatment-related complications" (Ilic et al., 2018).

In May of 2012, the USPSTF released a grade D recommendation against PSA prostate cancer screening (Ahlering et al., 2019). In 2018, the recommendation was switched to a grade C recommendation, now suggesting that men ages 55 to 69 could be screened for prostate cancer if first counseled about the benefits and harms of screening (USPSTF, 2018). Nonetheless, when the grade D recommendation was first released, many researchers were worried that an increase in late-stage prostate cancer cases would be identified, leading to greater rates of prostate cancer-specific mortality. To assess this risk, data from a total of 19,602 patients from nine high volume referral centers in the United States was collected and analyzed during the time that the USPSTF grade D recommendation was in effect. The researchers found that "All centers experienced consistent decreases of low-grade disease and absolute increases in intermediate and high-risk cancer. For any given age and PSA, propensity matching demonstrates more aggressive disease in the post-recommendation era" (Ahlering et al., 2019).

Osses et al. (2019) assessed the results of 1,134 men screened for prostate cancer in a 19 year follow up study; at the start of the study, all men were between the ages of 55 and 74. Unfortunately, 63% of the cohort was deceased by the 19-year follow-up period for various reasons. Still, the researchers noted that results suggested "a more substantial reduction in metastatic disease and cancer-specific mortality in favor of prostate cancer screening than previously reported" (Osses et al., 2019). However, more research needs to be completed with a larger sample size to confirm this conclusion.

Magnani et al. (2021) performed a cost analysis on "first-line prostate cancer management" using real-world data. A total of 3433 patients were included, and outcomes such as active surveillance (AS), surgery, and radiation were considered. Surgery was found to be the most common option, with 54.6% of the cohort compared to 22.3% for radiation and 23% for AS. Over a period of two years following diagnosis, AS was found to be the cheapest option at $2.97/day (d), with surgery costing $5.67/d and radiation costing $9.34/d, for "favorable" disease. For "unfavorable" disease, surgery cost $7.17/d and radiation cost $16.34/d. Over a period of five years following diagnosis, AS was found to be cheaper than surgery, by an amount of $2.71/d to $2.87 for surgery and $4.36 for favorable disease. For unfavorable disease, surgery remained cheaper than radiation, by an amount of $4.15/d to $10.32/d. The authors did remark that this information came from a single health care system and were based on benchmark Medicare estimates rather than actual payment exchanges (Magnani et al., 2021).

Baniak et al. (2020) compared the clinicopathologic and molecular characteristics of prostate cancer in 90 younger men (45 years or younger) to 200 men of typical screening age (60-65 years). The authors found that younger men tended to have lower PSA values, but a higher frequency of family history of

prostate cancer. No significant differences were found in staging or pathological characteristics of core biopsy specimens between the two groups. The younger cohort was also found to have a higher frequency of "grade group 1 disease" at radical prostatectomy. Finally, no statistically significant differences were found regarding prostatic adenocarcinoma specific recurrence/progression or death between the two cohorts (Baniak et al., 2020).

Guidelines and Recommendations

The American Association of Family Physicians (AAFP)

The AAFP recommends against the use of PSA-based testing for prostate cancer screening. For men between 55 to 69 years of age who are considering prostate cancer screening, the physician should discuss the risks and benefits and engage in shared decision making before undergoing the screening process. In addition, the AAFP recommends against prostate cancer screening in men older than 70 (AAFP, 2018b). They clarify that for men aged 55 to 69 "The decision to be screened for prostate cancer should be an individual one (Grade: C)", not to screen for prostate cancer in men aged 70 years and older (Grade: D). However, they also note that "Older age, African American race, and family history of prostate cancer are the most important risk factors for prostate cancer. The workup they describe involve starting with a test to measure PSA in the blood, and then positive results may be followed up with a transrectal ultrasound-guided core-needle biopsy of the prostate to diagnose prostate cancer (AAFP, 2018c).

The American Academy of Family Physicians (AAFP) and Choosing Wisely®

In collaboration with Choosing Wisely, the American Academy of Family Physicians has elaborated on some of their prostate cancer guidelines. There, they recommend against routine screening for prostate cancer using a prostate-specific antigen (PSA) test or digital rectal exam, but "For men who desire PSA screening, it should only be performed after engaging in shared decision making." Though screening may prevent mortality, "Whether this potentially small benefit in mortality outweighs the potential harms is dependent on the values and preferences of individual men. Therefore, for men who express a desire for prostate cancer screening, it should only be performed following a discussion of the potential benefits and harms. Routine screening for prostate cancer should not be done. PSA-based prostate cancer screening should not be performed in men over 70 years of age" (AAFP, 2018a).

The United States Preventive Services Task Force (USPSTF)

The USPSTF issued additional draft guidelines which recommend that clinicians inform men ages 55 to 69 years about the potential benefits and harms of PSA–based screening for prostate cancer, noting that the decision to be screened should be up to the patient. The USPSTF also states that screening offers a small potential benefit of reducing the chance of dying of prostate cancer. However, many men may be harmed due to false positives and its side effects such as overdiagnosis or other complications such as impotence and urinary incontinence. The USPSTF recommends discussion with a clinician before deciding to screen, ultimately giving this screening a "C" recommendation. Furthermore, the USPSTF recommends against PSA-based screening for prostate cancer in men over 70 (USPSTF, 2018). The CDC cites the USPSTF recommendation (CDC, 2022b).

The Memorial Sloan Kettering Cancer Center (MSK)

The Memorial Sloan Kettering Cancer Center issued screening guidelines on prostate cancer based on the individual's age. Their guidelines are for men who are expected to live at least ten years. "Men ages

45 to 49 should have a baseline PSA test. If the PSA level is 3 ng / mL or higher, men should talk with their doctor about having a biopsy of the prostate. If the PSA level is between 1 and 3 ng / mL, men should see their doctor for another PSA test every two to four years. If the PSA level is less than 1 ng / mL, men should see their doctor for another PSA test between the ages of 51 and 55. Men ages 50 to 59 should have their PSA level checked. If the PSA level is 3 ng / mL or higher, men should talk with their doctor about having a biopsy of the prostate. If the PSA level is between 1 and 3 ng / mL, men should see their doctor for another PSA test every two to four years. If the PSA level is less than 1 ng / mL, men should see their doctor for another PSA test at age 60. Men ages 60 to 70 should have their PSA level checked. If the PSA level is 3 ng / mL or higher, men should talk with their doctor about having a biopsy of the prostate. If the PSA level is between 1 and 3 ng / mL, men should see their doctor for another PSA test every two to four years. If the PSA level is less than 1 ng / mL, no further screening is recommended. Men ages 71 to 75 should talk with their doctor about whether to have a PSA test. This decision should be based on past PSA levels and the health of the man. Prostate cancer screening is not recommended for men ages 76 or older. A high PSA level does not generally mean that a man should have a prostate biopsy" (Memorial Sloan Kettering Cancer Center, 2022).

The National Comprehensive Cancer Network (NCCN)

The NCCN also recommends that patients make informed decisions regarding enrollment in an early detection program. Factors such as personal history, previous testing, family history, and race should be considered for determination if and when an early detection protocol is implemented. The guidelines stated that most panel members favored informed testing starting at 45. The panel supports screening in men until 75, and then continuing screening only in very healthy patients with little or no comorbidity to detect the life threatening and aggressive cancers. However, widespread screening in this age group is not recommended as it would increase rates of overdetection (NCCN, 2023b). The NCCN also relayed their concern about "the problems of overtreatment related to the increased frequency of diagnosis of prostate cancer from widespread use of PSA for early detection of screening" (NCCN, 2023a).

For initial testing, the NCCN recommends that "baseline PSA testing should be offered to healthy, well-informed men aged 45 to 75 years based on the results of RCTs" (NCCN, 2023b). They also recommend screening starting at 40 years for certain higher risk populations, such as those with "African ancestry", "suspicious family history", and germline mutations that increase risk of prostate cancer. Further, baseline testing may be ordered along with a DRE, and any elevated levels should be double checked with repeat testing (NCCN, 2023b).

The NCCN considers three categories for "early evaluation detection"; men of average risk (45-75 years), men of increased risk (such as men with "African ancestry," "suspicious family history," and "germline mutations that increase the risk of prostate cancer" [such as BRCA]), and men above 75 years.

- For men aged 45 to 75 years with average risk, the panel recommends repeat testing every 2 to 4 years if PSA is <1 ng/mL. If the PSA level is 1 to 3 ng/mL, frequency of testing should be every 1 to 2 years. If PSA > 3 ng/ml (or if the DRE is "very suspicious"), a biopsy should be considered.
- The above decision tree also applies to high-risk populations (African ancestry, suspicious or concerning family history, germline mutations that increase the rate of prostate cancer), though the NCCN recommends starting these evaluations at 40 years rather than 45 years of age. The NCCN also writes to "consider" screening these high-risk populations "annually" rather than the less frequent intervals discussed.
- For select patients over 75 years, repeat testing in select patients at 1- to 3-year intervals is recommended if the PSA is <4 ng/ml, the DRE is normal, and there are no other indications for

biopsy. In this case, they also state that one should "Consider discontinuing screening if clinically appropriate."

- However, in select patients over 75 years of age, if the patient has PSA >4 ng/ml and/or a very suspicious DRE, a repeat PSA test, DRE (if not performed during initial risk assessment), and workup for benign disease is indicated. A multiparametric MRI (mpMRI) should be considered. However, it is noted that "A negative MRI does not exclude the possibility of cancer", and so it is recommended to "Consider biomarkers and/or PSA density when deciding whether to avoid a biopsy in an individual with a negative mpMRI result."
    - If there is high suspicion for clinically significant cancer, image-guided biopsy via transrectal or transperineal approach with MRI targeting or without MRI targeting is indicated, though MRI targeting is preferred
    - If there is low suspicion for clinically significant cancer, there should be a follow-up in 6-12 months with PSA/DRE (NCCN, 2023b).

The NCCN also comments on several biomarkers' ability to assess early detection of prostate cancer.

They note that "Unbound or free PSA (fPSA), expressed as a ratio of tPSA, is a clinically useful molecular form of PSA, with the potential to improve early detection, staging, and monitoring of prostate cancer", explaining that "Most immunoreactive PSA is bound to the protease inhibitor alpha-1-antichymotrypsin. Other immunoreactive PSA-protease inhibitor complexes, such as alpha-1-antitrypsin and protease C inhibitor, exist at such low serum concentrations that their clinical significance has not been determined" (NCCN, 2023b). Another notable proportion of PSA is complexed with alpha-2-macroglobulin (AMG), though "this PSA-AMG complex cannot be measured by conventional assays because of the shielding (or "caging") of PSA antigenic epitopes by AMG" (NCCN, 2023b).

Testing for %fPSA is included in the NCCN guidelines as "an option before initial biopsy and for those with a prior negative biopsy" since "The FDA approved the use of %fPSA for the early detection of prostate cancer in individuals aged ≥50 years with a non-suspicious DRE and PSA levels between 4 ng/mL and 10 ng/mL (PSA levels where most secondary testing is done). The multi-institutional study that characterized the clinical utility of this assay showed that a 25% fPSA cutoff detected 95% of prostate cancers while avoiding 20% of unnecessary prostate biopsies" (NCCN, 2023b).

The NCCN also includes recommendations for PSA testing in non-screening situations, such as monitoring. Regarding "patients initially treated with intent to cure", the NCCN recommends testing serum PSA levels "every 6 to 12 months for the first 5 years and then annually." The NCCN also notes that for men with "high" risk of recurrence, testing PSA every three months may be preferred. For patients with castration-naïve disease on ADT [androgen deprivation therapy], PSA measurement may be done every three to six months based on clinical judgement. Moreover, the NCCN notes that "Local recurrence may result in substantial morbidity and can, in rare cases, occur in the absence of a PSA elevation. Therefore, annual DRE is appropriate to monitor for prostate cancer recurrence and to detect colorectal cancer. Similarly, after RT, the monitoring of serum PSA levels is recommended every six months for the first five years and then annually and a DRE is recommended annually. The clinician may opt to omit the DRE if PSA levels remain undetectable" (NCCN, 2023a).

The American Cancer Society (ACS)

The ACS recommends that physicians provide patients with information on benefits, risks, and uncertainties of the PSA test, and state that screening not be done until such information is received.

The ACS recommends that discussions (and screening) begin at age 50 for individuals of average risk, at age 45 for those at increased risk (i.e., "African American men and men who have a first-degree relative (father or brother) diagnosed with prostate cancer at an early age (younger than age 65)"), and at age 40 for those at highest risk (those with more than one first degree relative with a history of early-onset prostate cancer) (ACS, 2023a).

After having this discussion, individuals who want to be screened should get the PSA screening. A digital rectal exam (DRE) may also be performed. Because prostate cancer grows slowly, the ACS does not recommend PSA screening in any individual without symptoms of prostate cancer and with a life expectancy of less than 10 years. However, they also note that overall health and not age alone is important to decide when to pursue screening (ACS, 2023a).

If the initial PSA test is in normal range, the ACS recommends different testing intervals based on the initial test. For patients with results less than 2.5 ng/mL, the screening interval should be 2 years. For patients with initial results at or higher than 2.5 ng/mL, the screening interval should be annually (ACS, 2023a).

The ACS remarks that overall health and not age alone is important to decide when to pursue screening. Moreover, "Even after a decision about testing has been made, the discussion about the pros and cons of testing should be repeated as new information about the benefits and risks of testing becomes available" and further discussions must incorporate patients' health, values, and preferences (ACS, 2023a).

The National Cancer Institute (NCI)

The NCI has deemed the evidence insufficient to determine whether PSA-based screenings or DREs reduce mortality from prostate cancer. The NCI states that although screening can detect cancer in its earlier stages, it is unclear that earlier detection (and treatment) changes the natural course of the disease. The NCI also states that there is significant harm in screening such as overdiagnosis and complications caused by the screenings (NCI, 2023). Moreover, NCI states there is "solid evidence," that current treatments such as radical prostatectomy and radiation therapy, result in "permanent side effects in many men" (NCI, 2023).

The American College of Physicians (ACP)

The ACP agrees with the informed decision-making requirement for PSA testing, and states that clinicians should not screen using the PSA test in patients who "do not express a clear preference for screening." The ACP recommends that these discussions take place for men of average risk, ages 50 to 69 years. It is also worth mentioning that "it has been suggested that those who are at high risk may benefit from earlier screening beginning at age 45, while even higher risk men (those with two or more first-degree relatives with prostate cancer before age 65) should be screened at age 40." The ACP recommends against screening with PSA for individuals under 50 or over 70, as the primary target is between the ages of 50 and 69. The ACP also cautions that "Clinicians should not screen for prostate cancer using the PSA test in average-risk men aged 50 to 69 years who have not had an informed discussion and do not express a clear preference for screening" (Wilt et al., 2015). Asymptomatic men over 75 and those with a life expectancy of less than 10 years should also not be offered screening because screening can introduce substantial harms for questionable benefit (Qaseem et al., 2013; Wilt et al., 2015).

The American Urological Association (AUA)

The AUA recommends against use of PSA screening in men under 40, and routine screening for average-risk men between ages 40 to 54 years. The AUA does recommend informed decision making for men ages 55 to 69 years. The AUA recommends against PSA screening in men 70 years of age and older, or in any man with a life expectancy less than 10 – 15 years, although it is acknowledged that some men in excellent health 70 years and older may benefit from screening. The AUA recommends an individualized screening program be developed for individuals less than 55 years old, who are at high risk, such as those with a positive family history and African Americans. The AUA notes that a routine screening interval of two or more years may be preferred, but also notes that screening intervals "can be individualized by a baseline PSA level" (Carter et al., 2013).

The American Urological Association (AUA) and the Society of Urologic Oncology (SUO)

The American Urological Association and the Society of Urologic Oncology collaborated to produce a series of guideline statements. Regarding the use of PSA screening in the early detection of prostate cancer, they expounded that:

- "1. Clinicians should engage in SDM with people for whom prostate cancer screening would be appropriate and proceed based on a person's values and preferences. (*Clinical Principle*)"
- "2. When screening for prostate cancer, clinicians should use PSA as the first screening test. (*Strong Recommendation; Evidence Level: Grade A*)
- 3. For people with a newly elevated PSA, clinicians should repeat the PSA prior to a secondary biomarker, imaging, or biopsy. (*Expert Opinion*)"
- "4. Clinicians may begin prostate cancer screening and offer a baseline PSA test to people between ages 45 to 50 years. (*Conditional Recommendation; Evidence Level: Grade B*)
- "5. Clinicians should offer prostate cancer screening beginning at age 40 to 45 years for people at increased risk of developing prostate cancer based on the following factors: Black ancestry, germline mutations, strong family history of prostate cancer. (*Strong Recommendation; Evidence Level: Grade B*)"
- "6. Clinicians should offer regular prostate cancer screening every 2 to 4 years to people aged 50 to 69 years. (*Strong Recommendation; Evidence Level: Grade A*)"
- "7. Clinicians may personalize the re-screening interval, or decide to discontinue screening, based on patient preference, age, PSA, prostate cancer risk, life expectancy, and general health following SDM. (*Conditional Recommendation; Evidence Level: Grade B*)"
- "8. Clinicians may use digital rectal exam (DRE) alongside PSA to establish risk of clinically significant prostate cancer. (*Conditional Recommendation; Evidence Level: Grade C*)"
- "9. For people undergoing prostate cancer screening, clinicians should not use PSA velocity as the sole indication for a secondary biomarker, imaging, or biopsy. (*Strong Recommendation; Evidence Level: Grade B*)"
- "10. Clinicians and patients may use validated risk calculators to inform the SDM process regarding prostate biopsy. (*Conditional Recommendation; Evidence Level: Grade B*)"
- "11. When the risk of clinically significant prostate cancer is sufficiently low based on available clinical, laboratory, and imaging data, clinicians and patients may forgo near-term prostate biopsy. (*Clinical Principle*)" (Wei et al., 2023).

The AUA and SUO also partnered to discuss advanced prostate cancer as well, reported below (Lowrance et al., 2021).

Regarding the use of PSA screening for the Biochemical Recurrence without Metastatic Disease after Exhaustion of Local Treatment Options, they proposed that "Clinicians should inform patients with PSA

recurrence after exhaustion of local therapy regarding the risk of developing metastatic disease and follow such patients with serial PSA measurements and clinical evaluation. Clinicians may consider radiographic assessments based on overall PSA and PSA kinetics. *(Clinical Principle).*"

Moreover, PSA screening also plays a role in the prognosis of Metastatic Hormone-Sensitive Prostate Cancer, such that "Clinicians should obtain a baseline PSA and serial PSAs at three- to six-month intervals after initiation of ADT in mHSPC patients and consider periodic conventional imaging. *(Clinical Principle).*"

In patients with Non-Metastatic Castration-Resistant Prostate Cancer (nmCRPC), "clinicians should obtain serial PSA measurements at three- to six-month intervals, and calculate a PSADT starting at the time of development of castration-resistance. *(Clinical Principle).*"

Lastly, in patients with Metastatic Castration-Resistant Prostate Cancer, "clinicians should obtain baseline labs (e.g., PSA, testosterone, LDH, Hgb, alkaline phosphatase level) and review location of metastatic disease (lymph node, bone, visceral), disease-related symptoms, and performance status to inform discussions of prognosis and treatment decision-making. *(Clinical Principle).*" However, "In mCRPC patients without PSA progression or new symptoms, clinicians should perform imaging at least annually. *(Expert Opinion)*" (Lowrance et al., 2021).

The European Society for Medical Oncology (ESMO)

The ESMO recommends against population-based screening for prostate cancer because the reduction in mortality does not offset the harms done, such as overdiagnosis and overtreatment. Early PSA testing should only be offered to men > 50 years, men > 45 years with a family history of prostate cancer, African Americans > 45 years, and *BRCA1/2* carriers who are > 40 years of age. Prostate cancer screening should not be performed in asymptomatic men with a life expectancy of less than ten years. ESMO also recommends against screening in asymptomatic men over 70 (Parker et al., 2020).

The American Association of Clinical Urologists Inc. (AACU)

The AACU recommends use of tissue-based molecular testing to assess risk stratification in prostate cancer treatment decision making. The AACU states pursing germline testing when appropriate is encouraged and support any further research into these tests (AACU, 2018).

The European Association of Urology (EAU)

In 2021, the EAU released updated guidelines on their position regarding prostate antigen testing, recommending a risk-adapted strategy for the early detection of prostate cancer and reaffirming the joint guidelines in the table above. They noted that an absence of regular and routine widespread PSA testing had led to "opportunistic" testing in several EU member states. In addition, the impact of COVID-19, particularly the redeployment of medical resources to fight COVID-19, as well as a move during COVID-19 to "deprioritise all oncology screening, including PSA testing," was of strong concern. Their position concluded with the need to reverse current unfavorable trends in order to accurately diagnose advanced stage prostate cancer and save lives (Van Poppel et al., 2021).

The European Association of Urology (EAU), European Association of Nuclear Medicine (EANM), European Society for Radiotherapy and Oncology (ESTRO), European Society of Urogenital Radiology (ESUR), and International Society of Geriatric Oncology (SIOG)

Joint guidelines on prostate cancer screening and early detection from the EAU, EANM, ESTRO, ESUR, and SIOG include the table below taken from Mottet et al. (2020).

Recommendation	Strength Rating
Do not subject men to prostate-specific antigen (PSA) testing without counselling them on the potential risks and benefits.	Strong
Offer an individualised risk-adapted strategy for early detection to a well-informed man with a good performance status (PS) and a life-expectancy of at least ten to fifteen years.	Weak
Offer early PSA testing in well-informed men at elevated risk of having PCa: **men > 50 years of age;** **men > 45 years of age and a family history of PCa;** **African-Americans > 45 years of age.** **Men carrying *BRCA2* mutations > 40 years of age**	Strong
Offer a risk-adapted strategy (based on initial PSA level), with follow-up intervals of two years for those initially at risk: **men with a PSA level of > 1 ng/mL at 40 years of age;** **men with a PSA level of > 2 ng/mL at 60 years of age;** **Postpone follow-up to eight years in those not at risk.**	Weak
Stop early diagnosis of PCa based on life expectancy and performance status; men who have a life-expectancy of < fifteen years are unlikely to benefit.	Strong

Additional guidelines for the risk-assessment of asymptomatic men from (Mottet et al., 2020) state:

Recommendation	Strength rating
To avoid unnecessary biopsies, offer further risk-assessment to asymptomatic men with a normal digital rectal examination (DRE) and a prostate-specific antigen (PSA) level between 2-10 ng/mL prior to performing a prostate biopsy. Use one of the following tools: **risk-calculator;** **imaging;** **an additional serum or urine-based test (weak strength rating)**	Strong

The guideline also notes the presence of newer biological markers, such as "TMPRSS2-ERG fusion, PCA3, or kallikreins as incorporated in the Phi or 4Kscore tests" but despite promising early results, the guideline considers these markers to have "too limited data to implement these markers into routine screening programmes" (Mottet et al., 2020).

Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration

The FDA has approved several screening tests for prostate cancer beginning with a PSA immunoassay in 1986 (FDA, 2023).

On June 14, 2012, the FDA approved the Access® Hybritech® p2PSA assay created by Beckman Coulter, Inc. From the FDA website: "The Access® Hybritech® p2PSA assay is a paramagnetic particle, chemiluminescent immunoassay for the quantitative determination of [-2] proPSA antigen, an isoform of free PSA, in human serum using the Access Immunoassay Systems. Access@ Hybritech® p2PSA is intended to be used in combination with Access® Hybritech® (total) PSA and Access@ Hybritech@ free PSA to calculate the Beckman Coulter Prostate Health Index (phi), an In Vitro Diagnostic Multivariate Index Assay (IVDMIA)" (FDA, 2012).

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
84152	Prostate specific antigen (PSA); complexed (direct measurement)
84153	Prostate specific antigen (PSA); total
84154	Prostate specific antigen (PSA); free
G0103	Prostate cancer screening; prostate specific antigen test (PSA)

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

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Revision History

Revision Date	Summary of Changes
03/06/2024	Reviewed and Updated: Updated the background, guidelines and recommendations, and evidence-based scientific references. Literature review did not necessitate any modifications to coverage criteria. The following edits were made for clarity: Removed TRUS-guided biopsy and DRE from CC5, as these are outside Avalon's scope of management. CC 5 now reads: "5) A percent free PSA test or a follow-up in 6-12 months with total PSA MEETS COVERAGE CRITERIA when any of the following conditions are met:" Addition of "prostate cancer" to CC7.d. for clarity. Now reads: "d) For monitoring response to prostate cancer therapy."
03/01/2023	Reviewed and Updated: Updated the background, guidelines and recommendations, and evidence-based scientific references. Literature review did not necessitate any modifications to coverage criteria. The following edits were made for clarity:

	All CC edited for clarity and consistency. CC4 was split into two coverage criteria for consistency in coverage criteria formatting. Now read: "4) For individuals with previous total PSA results, repeat screening for prostate cancer with a total PSA test MEETS COVERAGE CRITERIA with the following frequency: a) For individuals less than 76 years of age, when total PSA is <1 ng/ml and digital rectal exam (DRE) is normal (if done): Repeat screening at 2- to 4-year intervals. b) For individuals less than 76 years of age, when total PSA is 1-3 ng/ml and DRE is normal (if done): Repeat screening at 1- to 2-year intervals. c) For individuals greater than 75 years of age, when total PSA is <4 ng/ml and DRE is normal (if done) and no other indications for biopsy: Repeat screening in select patients (very healthy individuals with little or no comorbidity) at 1- to 4- year intervals. 5) TRUS-guided biopsy, percent free PSA, or a follow-up in 6-12 months with total PSA or DRE MEETS COVERAGE CRITERIA when any of the following conditions are met: a) For individuals less than 76 years of age with a total PSA >3 ng/ml and/or a very suspicious DRE. b) For select individuals greater than 75 years of age (very healthy individuals with little or no comorbidity) with a total PSA >4 ng/ml or a very suspicious DRE."
03/09/2022	Annual review: Updated background, guidelines and recommendations, and evidence-based scientific references. Literature review did not necessitate modification to coverage criteria. CPT Changes - The indicator for code 84152 changed from "PA Not Req." to "Not Covered"
03/03/2021	Annual review: Updated background, guidelines and recommendations, and evidence-based scientific references. Policy Title was changed from "Prostate Cancer Screening" to "Prostate Specific Antigen (PSA) Testing. Literature review necessitated changes to the following coverage criteria: Per 2021 NCCN (V.1, Prostate Cancer), the CC concerning the age range of 45-75 was more clearly defined to mention "average-risk" and "all" was removed: • Screening for prostate cancer with the prostate-specific antigen (PSA) test MEETS COVERAGE CRITERIA for average-risk individuals aged 45-75 years Per 2021 NCCN (V.1, Prostate Cancer), a CC was added concerning screening age ranges for higher-risk populations, such as individuals with a suspicious family history: • Screening for prostate cancer with the prostate-specific antigen (PSA) test annually MEETS COVERAGE CRITERIA for individuals aged 40-75 years with: a. African ancestry b. Germline mutations that increase risk for prostate cancer c. Suspicious family history The CCs regarding individuals >75 years and PSA testing beyond screening were reworded for clarity by adding "for prostate cancer" and stated as separate CC: • For individuals over 75 years, screening for prostate cancer with a PSA test MEETS COVERAGE CRITERIA only for individuals with little or no comorbidities. (*See Note 1

	below) In the CC about repeat screening for prostate cancer, in the age category 45-75, "45" was removed for clarity since NCCN v.1.2021 allows screening from 40 years for high-risk groups. Following CC was removed because this statement was reworded per NCCN v.1.2021 in CC2: • PSA testing annually MEETS COVERAGE CRITERIA for individuals with signs or symptoms of prostate cancer or in high-risk populations (eg. African-American individuals and individuals with a family history of prostate cancer or individuals with a presence of inherited mutations). Added from STM AHS-G2124 policy "and for monitoring response to therapy, and detecting disease recurrence" and "for initial prostate cancer diagnosis": • PSA testing MEETS COVERAGE CRITERIA for initial prostate cancer diagnosis in individuals with signs and symptoms of prostate cancer (See Note 2), for follow-up of individuals with a current or previous diagnosis of prostate cancer, and for ongoing monitoring of individuals who have undergone tumor resection or prostatectomy, and for monitoring response to therapy, and detecting disease recurrence. Removed this wording "4Kscore, Prostate Health Index (PHI), PCA3 score or ConfirmMDx" from the following CC and moved it to AHS-M2166 policy: • Use of percent free PSA as a first-line screening test for prostate cancer Removed following CC from this policy and moved it to AHS-M2166 policy: • Other screening tests for prostate cancer, including, but not limited to, alpha-methylacyl coenzyme A racemase (AMACR), early prostate cancer antigen, endoglin, E twenty-six (ETS) gene fusions, human kallikrein 2, analysis of prostatic fluid electrolyte composition, interleukin-6, transforming growth factor-beta 1, TMPRSS2:ERG gene fusion, and gene hypermethylation, DO NOT MEET COVERAGE CRITERIA because their clinical utility has not been proven. • Other tests using cellular and biologic features of a tumor DO NOT MEET COVERAGE CRITERIA, including use in predicting risk of recurrence in patients with prostate cancer. Added per NCCN v.1.2021 "or have a rising PSA" to the NOTE1: NOTE 1: According to the NCCN guidelines, "Testing after 75 years of age should be done only in very healthy men with little or no comorbidity (especially if they have never undergone PSA testing or have a rising PSA) to detect the small number of aggressive cancers that pose a significant risk if left undetected until signs or symptoms develop. Widespread screening in this population would substantially increase rates of over-detection and is not recommended" (NCCN, 2021) (NCCN, 2018, 2021). Additionally, the term individuals in this policy apply to individuals who have a prostate or were born with a prostate. Word "total" was added to PSA test for clarity. Added NOTE 2 to clarify signs and symptoms:

	NOTE 2: According to ACS, 2019: "Most prostate cancers are found early, through screening. Early prostate cancer usually causes no symptoms. More advanced prostate cancers can sometimes cause symptoms, such as: • Problems urinating, including a slow or weak urinary stream or the need to urinate more often, especially at night • Blood in the urine or semen • Trouble getting an erection (erectile dysfunction or ED) • Pain in the hips, back (spine), chest (ribs), or other areas from cancer that has spread to bones • Weakness or numbness in the legs or feet, or even loss of bladder or bowel control from cancer pressing on the spinal cord (ACS, 2019)."
03/10/2020	Annual review: Updated background, guidelines and recommendations, and evidence-based scientific references. Removed Note 2 of the CCs regarding genetic testing since it is no longer pertinent to this policy. The GEP testing is now in AHS-M2166. Changed E&I CCs to DNMCC with a preceding statement regarding a lack of published scientific literature. Literature review did not necessitated any additional modifications to the coverage criteria. For clarity, replaced 'testing' with 'screening' in CC regarding repeat screening for prostate cancer. For clarity, reworded CC from "Other tests utilizing systems pathology that uses cellular and biologic features of a tumor…" to "Other tests using cellular and biologic features of a tumor…"
06/20/2019	Off-cycle Review: Removed the following and placed in new policy AHS-M2166 Gene Expression Profiling and Protein Biomarkers for Prostate Cancer Management: • The one-time use of Prolaris®, Oncotype DX®, Promark®, OR Decipher® tumor-based molecular assays to guide management of prostate cancer **MEETS COVERAGE CRITERIA** only if ALL the following criteria have been met: o Needle biopsy with localized adenocarcinoma of prostate with no clinical evidence of metastasis or lymph node involvement; AND o No presence of significant co-morbidities, including advanced age, to suggest individual has an estimated life expectancy of less than 10 years; AND o Patient must fall into one of the following stages, as defined by the NCCN* (See Note 2): ▪ Low Risk ▪ Favorable Intermediate Risk • Urine testing for gene expression profile to screen for prostate cancer or to assess prostate cancer as **E&I**. • Prostate cancer gene 3 (PCA3) as **E&I.** • PCA3/KLK3 (prostate cancer antigen 3 [non-protein coding]/kallikrein-related peptidase 3 [prostate specific antigen]) ratio (eg, prostate cancer) as **E&I.** Coding review: Removed 0005U, 0047U and 81541. Added 0021U, 0053U.
03/01/2019	Annual review: Updated definition, background, guidelines & recommendations, and evidence-based scientific references. Per 2018 NCCN guidelines, the following changes were made:

	▪ Added CC for one-time use of Prolaris, Oncotype DX, Promark, or Decipher tumor-based molecular assays to guide management of prostate cancer **MCC** for specific populations and circumstances ▪ Added "Urine testing for gene expression profile to screen for prostate cancer or to assess prostate cancer" to the list of **E&I** ▪ Removed the wording "and genetic testing" from **E&I**; however, left gene fusions and gene hypermethylation testing as **E&I**. This is to prevent apparent contradiction with new CC. ▪ Removed CC subpoint stating "Gene expression analysis to guide management of prostate cancer is **EXPERIMENTAL** and **INVESTIGATIONAL**." ▪ Added Note 1 from NCCN guidelines regarding PSA testing in men over the age of 75 and term "individuals" was specified ▪ Added Note 2 from NCCN guidelines consisting of table outlining recommendation concerning molecular testing The term "men" was replaced with "individuals" specifying in Note 1 to whom it applies Added PLA codes 0005U and 0047U.
12/13/2018	Off cycle review: CPT code 88399 removed from 2019 PA List with disposition of APEA permissible.
7/11/2018	Off Cycle review: CPT code 81539 changed to not covered as it is not on the 2018 PA list.
03/16/2018	Annual review: Definitions, Background, Guidelines and Recommendations, Federal Regulations and Evidence-based Scientific References were updated. Literature review did necessitate modification to coverage criteria: CC2 D&E per NCCN v.2.2017; CC4 addition of annual testing for high-risk populations per NCCN v.2.2017 No changes in coding.
01/1/2018	Off cycle review: Adding new 2018 codes 81541 and 81551 as not covered
08/30/2017	Off cycle review: Added CPT code 84066
03/20/2017	Annual review: -Updated reference sections. Coverage criteria # 2 updated - American Cancer Society recommends annual testing for PSA greater or equal to 2.5 ng/ml. Link. CPT code 81539 changed to not covered from PA required, as sole use for this code governed by this policy.
09/28/2016	Off cycle review. Added CPT 81599 as PA required
02/26/2016	Off cycle review. Policy content updated to reflect that 4Kscore does not meet coverage criteria.
06/16/2015	Initial Presentation

Salivary Hormone Testing

Policy Number: AHS – G2120 – Salivary Hormone Testing	Prior Policy Name and Number, as applicable: G2120 – Salivary Hormone Testing for Menopause and Aging
Initial Presentation Date: 09/18/2015 Revision Date: 03/06/2024	

Policy Description

Testing of saliva has been proposed as a noninvasive method to measure free (unbound to carrier proteins) steroid hormones, including estrogen, progesterone, androgens, and cortisol, for diagnosis of hormonal imbalance and administration of individualized hormone replacement therapy (ACOG & ASRM, 2012).

Hypercortisolism can occur in several disorders, including Cushing syndrome (pituitary hypersecretion of corticotropin/ACTH), or glucocorticoid administration resulting in obesity, hypertension, menstrual irregularity, and glucose intolerance (Lacroix et al., 2015; Nieman et al., 2008; Nieman, 2022; Quddusi et al., 1998).

Terms such as male and female are used when necessary to refer to sex assigned at birth.

Related Policies

Policy Number	Policy Title
AHS-G2013	Testosterone

Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) For individuals with signs and symptoms of Cushing syndrome, late night salivary cortisol testing **MEETS COVERAGE CRITERIA**.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

2) For the screening, diagnosis, **and/or** monitoring of menopause, infertility, endometriosis, polycystic ovary disease (PCOS), premenstrual syndrome, osteoporosis, sexual dysfunction, seasonal affective disorder, depression, multiple sclerosis, sleep disorders, **or** diseases related to aging, salivary hormone testing **DOES NOT MEET COVERAGE CRITERIA**.

Table of Terminology

Term	Definition
AACE	American Association of Clinical Endocrinologists
ACOG	American College of Obstetricians and Gynecologists
ACTH	Adrenocorticotropic hormone
ASRM	American Society of Reproductive Medicine Practice Committee
CLIA '88	Clinical Laboratory Improvement Amendments of 1988
CMS	Centers for Medicare and Medicaid Services
CS	Cushing syndrome
DHEA	Dehydroepiandrosteron
E1	Estrone
E2	Estradiol
E3	Estriol
EIA	Enzyme immunoassay
ELISA	Enzyme-linked immunosorbent assay
ERCUSYN	European Registry on Cushing's Syndrome
ES	Endocrine Society
FDA	Food and Drug Administration
HT	Hormone therapy
IVF	In vitro fertilization
LC-MS	Liquid chromatography with tandem mass spectrometry
LDTs	Laboratory-developed tests
LNSC	Late night salivary cortisol
MHT	Menopausal hormone therapy
MP	Micronized progesterone
MS	Multiple sclerosis

NAMS	North American Menopausal Society
PCOS	Polycystic ovary disease
RIA	Radioimmunoassay
TSS	Transsphenoidal selective adenomectomy

Scientific Background

Testing of hormone levels in the saliva has been proposed as a noninvasive method to measure free (unbound to carrier proteins and thus active) steroid hormones (estrogen, progesterone, androgens, cortisol, etc.) for diagnosis of hormonal imbalance and administration of individualized hormone replacement therapy (ACOG & ASRM, 2012). Saliva measurements are thought to represent the concentrations of unconjugated steroid hormones as well as unconjugated steroids that have diffused freely into saliva. Conjugated steroids will often show significant decreases in concentration because their filtration process into the saliva is limited. This is what causes hormones, such as cortisol, estradiol, and testosterone to approximate concentrations well and the hormone dehydroepiandrosterone (DHEA) to represent concentrations poorly (Wood, 2009).

Salivary hormone level testing is often recommended by bioidentical hormone vendors as a means of providing personalized therapy. However, individualized testing and monitoring is only useful when a narrow therapeutic window exists for a drug or a drug class. Steroid hormones, such as estrogen and progesterone, do not meet these criteria and do not require individualized testing (ACOG & ASRM, 2012; Conaway, 2011). Furthermore, there is no evidence that hormonal levels in saliva are biologically meaningful. Saliva is an ultra- filtrate of the blood and in theory, should be amenable to testing for free concentrations of hormones; however, salivary testing does not appear to be an accurate or precise method of hormone testing (Flyckt et al., 2009; Lewis et al., 2002). Studies suggest that salivary assessments of hormone levels are inaccurate and do not correlate with levels determined from serum (Conaway, 2011), as there is large within-patient variability in salivary hormone concentrations, especially when exogenously administered hormones are given (Hardiman et al., 1990; Klee & Heser, 2000; Lewis et al., 2002; Meulenberg et al., 1987; Wren et al., 2000). Salivary hormone levels often fluctuate with factors, such as circadian rhythm, and frequently do not correlate well with serum levels of hormones (Wood, 2009).

Salivary hormone measurement may be utilized for many purposes. Menopause occurs due to changing hormone levels, mainly estrogen. In general, [individuals] experience menopause at a mean age of 51 years, with most becoming menopausal between 45 and 55. Menopausal hormone therapy (MHT, estrogen alone or combined with a progestin) is used for management of menopausal symptoms and is highly effective for symptoms, such as hot flashes and vaginal atrophy. In some cases, MHT may be used for the mood lability that many [individuals] experience during the menopausal transition (Martin & Barbieri, 2022; Taylor & Manson, 2011). There are few indications for the measurement of hormone levels to evaluate success of therapy when treating a postmenopausal [individual] with hormones. If treatment is initiated for symptom control, therapy should be titrated to the alleviation of symptoms, not a laboratory value (ACOG & ASRM, 2012). A salivary hormone test has been developed by Genova Diagnostics, which evaluates levels of hormones in [individuals] during perimenopause, menopause, and andropause (Genova Diagnostics, 2020).

One of the primary hormones that diffuses freely into saliva and can be well-approximated by salivary measurements is cortisol. Cortisol is a steroid hormone that is produced due to stress. Salivary flow rate does not affect cortisol concentration, and salivary cortisol correlates well with serum-free cortisol. This

property can be used to identify adrenal insufficiencies and other related disorders (Nieman, 2019). For example, the presence of Cushing syndrome (CS) is suggested by signs of hypercortisolism, such as proximal myopathy, facial plethora, and wide purplish striae. However, none of these are pathognomonic, and many are nonspecific (such as obesity or hypertension). As a result, the diagnosis must be confirmed by biochemical tests, one of which is a salivary cortisol measurement (Nieman, 2023). The recurrence of hypercortisolemia after an initial treatment for CS seems to be predicted earlier by late night salivary cortisol (LNSC) testing compared to urinary free cortisol excretion (Fleseriu et al., 2016).

Proprietary Testing

Saliva testing measures the amount of hormone available to target tissues and is a good option for monitoring hormonal therapy. ZRT Laboratories developed a Saliva Steroid Profile using liquid chromatography/tandem mass spectrometry (LC-MS/MS) which tests a broad range of bioavailable hormones and hormone metabolites in one saliva sample collection. LC-MS/MS testing accurately reports levels of estrogen, such as those seen in men, children, and people using aromatase inhibitors, and includes a test for ethinyl estradiol, three hormone blockers, and melatonin. "Testing the levels of both upstream precursors and downstream metabolites of these parent active steroids [estrogens, progestogens, androgens, glucocorticoids, mineralocorticoids, melatonin, synthetic estrogen ethinyl, estradiol, anastrozole, letrozole, and the 5α-reductase inhibitor finasteride] will help determine which steroid synthesis enzymes are low, overactive, blocked by natural or pharmaceutical inhibitors, or defective due to metabolic dysfunctions (e.g., Polycystic Ovarian Syndrome (PCOS), Premenstrual Dysphoric Disorder (PMDD), luteal dysfunction, overexpression of aromatase, and estrogen dominance) and inborn errors of metabolism such as Congenital Adrenal Hyperplasia (CAH)" (ZRTLAB, 2019). ZRT is one of the first labs to measure hormones in saliva and helped establish the method that made saliva hormone testing commercially viable for health care providers and patients around the globe.

UnikeyHealth developed a saliva hormone testing panel to assess six hormone levels with an at-home test. The hormones tested are progesterone, estradiol, estriol, testosterone, DHEA, and cortisol. This at-home test provides recommendations and is purported to identify underlying causes of hormonal imbalance issues based on the individualized hormone assessment (UnikeyHealth, 2022).

Genova Diagnostics has developed several saliva hormone tests including The Rhythm™ hormone test (Genova, 2022c), Menopause Plus™ (Genova, 2022b), The Comprehensive Melatonin Profile (Genova, 2022a), and The Adrenocortex Stress Profile (Genova, 2023). The Rhythm™ hormone test is a comprehensive assessment of estradiol, progesterone, and testosterone which can help assess underlying causes of disorders such as premenstrual syndrome (PMS), infertility, and menstrual irregularities (Genova, 2022c). Menopause Plus™ is Genova's most comprehensive salivary hormone profile and is designed to provide insight into the impact that shifting hormone levels may play in men (andropause or male menopause) and women (peri/menopause). This test collects eight saliva samples every other day over six days for estrone (E1), estradiol (E2), estriol (E3), progesterone, progesterone/estradiol ratio (P/E2), and testosterone (Genova, 2022b). The Comprehensive Melatonin Profile analyzes the circadian secretion patterns of melatonin by analyzing three saliva samples taken in the morning, afternoon, and midnight. This test is purported to determine underlying causes of melatonin imbalances in sleep disorders, depression, and seasonal affective disorder (Genova, 2022a). Lastly, The Adrenocortex Stress Profile (ASP) provides an assessment of the Hypothalamic-Pituitary-Adrenal (HPA) axis using carefully timed salivary samples of cortisol and DHEA. This may help reveal HPA axis imbalances which could be a contributing factor in cardiovascular disease, immune dysregulation, diabetes, chronic fatigue, persistent pain, or cognitive decline (Genova, 2023).

Analytical Validity

Multiple proprietary tests are available for salivary hormone testing. Tests such as ZRT and UnikeyHealth ask the user to submit saliva samples and send the specimen to the proprietary lab where it can be analyzed. Labs will typically use an immunoassay-based method, such as an enzyme-linked immunosorbent assay (ELISA) or enzyme immunoassay (EIA), to assess the concentration of hormones, such as estradiol or progesterone. Others may use an automated competitive electrochemiluminescence immunoassay for LNSC measurement (Spence et al., 2018). The results are compiled into a report listing the concentrations of each hormone as well as comments on abnormal amounts. These tests are often marketed to post-menopausal [individuals] who desire to have an assessment of hormones like estrogen, progesterone, DHEA, testosterone, estriol, and cortisol (UnikeyHealth, 2022; ZRTLAB, 2019). Moreover, another proprietary test proposes that conditions such as multiple sclerosis (MS) can be assessed through irregularities in melatonin (Genova, 2022a). However, not only is melatonin not widely measured through saliva, but there is currently no compelling data for whether administering melatonin has any utility with dealing with MS; there has been far too little published data with human subjects to draw any conclusions (Wurtman, 2017). Osteoporosis is another condition that tests may purportedly be able to screen for with saliva (Genova, 2022c). However, this test may be of limited utility as the risks of hormone therapy may outweigh the benefits (Rossouw et al., 2002).

Salivary cortisol was first measured by direct radioimmunoassay (RIA) in 1978, but more accurate cortisol immunoassays have now been developed; however, these assays are often limited due to poor specificity (El-Farhan et al., 2017). Further, late at night, cortisol levels may fall below detection limits for some RIA testing methods. Liquid chromatography with tandem mass spectrometry (LC-MS-MS) has also been used for the detection of salivary cortisol. Schiffer et al. (2019) developed a novel LC-MS/MS assay to identify androgens in saliva samples with appropriate sensitivity. Prior, Li et al. (2018) was able to utilize the same technique (LC-MS/MS) to accurately quantify three estrogens (estrone E1, estradiol E2, and estriol E3) in an assay with an accuracy of 98.9-112.4% and precision of (≤7.4%) as a hopeful alternative to blood samples. However, this field continues to face limitations due to poorly standardized assays and a lack of a single, validated reference range (El-Farhan et al., 2017).

Initial diagnostic tests for hypercortisolism should be highly sensitive, even if the diagnosis may be excluded later. Late night salivary cortisol (LNSC) is a first-line diagnostic test for CS as indicated by the approach outlined by the 2008 Endocrine Society (Nieman et al., 2008) and others (Hinojosa-Amaya et al., 2019). LNSC measurements are obtained at least twice because the hypercortisolism in CS may be variable. Two measurements must be abnormal for the test to be considered abnormal; this may be especially difficult for patients with fluctuating disease. The diagnosis of CS is established when at least two different first-line tests (such as LNSC and 24-hour urinary cortisol excretion) are abnormal. Once the diagnosis is established, additional evaluation is done to identify the cause of the hypercortisolism (Nieman, 2023).

A locally modified RIA assay was developed by Nunes et al. (2009) and measured LNSC in obese patients with a current or past diagnosis of CS. The assay was able to diagnose a recurrence of CS with a sensitivity of 90% and a specificity of 91.8%; it was also reported that "A threshold of 12 nmol/liter yielded 100% sensitivity and specificity in overt [Cushing] syndrome" (Nunes et al., 2009).

Ueland et al. (2021) studied the analytical validity of late-night salivary cortisol as a screening test for CS. Bedtime and morning salivary samples were collected from 54 children in the obesity clinic and three children with pituitary CS using liquid chromatography tandem mass spectrometry (LC-MS/MS). These levels were compared to 320 salivary samples from healthy children to set cut-off values. Bedtime cutoff

levels for cortisol and cortisone were 2.4 and 12.0 nmol/L, respectively. By "Applying these cutoff levels on the verification cohort, 1 child from the obesity clinic had bedtime salivary cortisol exceeding the defined cutoff level, but normal salivary cortisone. All 3 children with pituitary CS had salivary cortisol and cortisone far above the defined bedtime cutoff levels. Healthy subjects showed a significant decrease in salivary cortisol from early morning to bedtime" (Ueland et al., 2021). The authors conclude that bedtime salivary cortisol levels with a diagnostic threshold above 2.4 nmol/L can be applied as a screening test for CS in children.

Clinical Utility and Validity

A study by Lewis et al. (2002) focusing on salivary progesterone measurements found major variation when a progesterone cream was applied to several post-menopausal [individuals]. Salivary measurements were collected at zero, one, three, four, seven, and eight weeks. The average baseline for the 20 mg/g cream group was found to be 0.25 ± 0.12 nmol/L, but the measurement at one week was 82.11 ± 104.52 nmol/L (Lewis et al., 2002); similar enormous variations were found at three and seven weeks, as well as the 40 mg/gm cream group. In contrast, the placebo group's baseline was 0.43±0.21 and 0.38±0.20 in week eight (Lewis et al., 2002). The finding with inconsistent salivary progesterone levels was even found among premenopausal [individuals] obtaining *in vitro* fertilization (IVF); on the other hand, salivary estradiol was found to be correlative to serum-based assessment, and could be a less invasive alternative to blood draws for ovarian stimulation during IVF cycles (Sakkas et al., 2020).

Late night salivary cortisol (LNSC) measurements were found to be concordant with the 24-hour urine test, with 97% concordance at ≥4 nmol/L and 69% concordance at ≥ 10 nmol/L. However, the tests were stated to be "equivalent" at the more sensitive cutoff of 4 nmol/L. The authors concluded that due to the concordance of the salivary test with the urine test, the salivary test should replace the urinary test as the frontline test for Cushing syndrome (Doi et al., 2013). Another study found LNSC to be 100% sensitive and 98% specific at a cut-off of 2.4 nmol/L (Antonelli et al., 2015). Both cortisol and its metabolite cortisone were tested as cortisone is a significant source of interference in certain immunoassays. The variation between and within runs were both under ten percent, the method was linear up to 55.4 nmol/L for cortisol, and the lower of limit of quantification was 0.51 nmol/L for cortisol (Antonelli et al., 2015).

A study measured the utility of salivary testosterone and cortisol concentrations in 71 junior athletes (26 females and 45 males) in response to stress. The researchers compared results of salivary samples to capillary blood samples taken at the same time; while blood samples showed an increase in both testosterone and cortisol concentrations in both sexes, salivary samples showed no change in testosterone or cortisol levels (Crewther et al., 2018). This may suggest that salivary hormone testing in these populations is not as efficient as other methods.

Valassi et al. (2017) analyzed diagnostic data from 1341 CS patients in the European Registry on Cushing's syndrome (ERCUSYN) and noted that of the three main first-line CS diagnostic tests, the urinary free cortisol test was performed in 78% of patients as a first-line testing method, overnight 1 mg dexamethasone suppression test was performed in 60% of patients, and LNSC was performed in only 25% of patients. This shows that LNSC may not be used as frequently as other testing methods for a first-line diagnosis of CS.

Salivary testing for cortisol could also prove useful in occupational settings as a parameter for stress. Oldenburg and Jensen (2019) conducted a study on merchant ship crew, and found that after adjustment, average salivary cortisol level was positively associated with "acute shipboard stressors,

namely the average current working time (p=.050) and the average number of terminals that had been served during the last 7 days (p=0.008)." This laboratory data is essential in all fields wherein professionals experience high levels of stress, so that measures can be taken to create a positive working environment.

Kim et al. (2020) studied the diagnostic utility of stimulated salivary cortisol as a noninvasive diagnostic tool for adrenal insufficiency (AI). One hundred twenty subjects were measured for stimulated cortisol levels and these levels were compared to those obtained from the short Synacthen test (SST). AI was defined as a cortisol level of <496.8 nmol/L during the SST. Thirty-four of 120 patients were diagnosed with AI according to SST results. "Basal and stimulated salivary cortisol levels were positively correlated with basal (r=0.538) and stimulated serum cortisol levels (r=0.750), respectively (all P<0.001)." The cut off level of morning basal salivary cortisol was 3.2 nmol/L, and the cutoff value of stimulated salivary cortisol was 13.2 nmol/L. Subjects with a stimulated salivary cortisol level above 13.2 nmol/L but a stimulated serum cortisol level below 496.8 nmol/L (n= 2) had lower serum albumin levels than those showing a concordant response. The authors conclude that "The diagnostic performance of stimulated salivary cortisol measurements after the SST was comparable to serum cortisol measurements for diagnosing AI" (Kim et al., 2020).

Guidelines and Recommendations

American Association of Clinical Endocrinologists (AACE)

The AACE has noted salivary hormone level testing as recommended by certain proponents to provide individualized therapy. However, these methods are not FDA or CLIA approved, and factors such as hydration and circadian rhythm may influence the concentration of hormones within a subject. Standardization is difficult, and even though standardized blood tests do exist; it is of limited clinical utility because measuring hormone levels in postmenopausal [individuals] has no predictive value on what the normal levels should be. A salivary measurement cannot be used to correct the levels of sex hormones (Goodman et al., 2011).

American College of Obstetricians and Gynecologists (ACOG) and the American Society of Reproductive Medicine Practice Committee (ASRM)

American College of Obstetricians and Gynecologists and the American Society of Reproductive Medicine Practice Committee released joint guidelines on compounded hormone therapy that stated salivary hormone testing had no evidence to support its biological utility and that testing the hormone levels were neither accurate nor precise. The guidelines stated that salivary hormone testing had large intra-patient variability depending on factors such as diet and that saliva did not provide a reasonable representation of serum hormone levels. Saliva may be contaminated with other cell types, contains lower concentration of hormones than serum, and impossible to reliably test for a representative result. The guidelines concluded that evidence is inadequate to support an individualized hormone therapy based on salivary, serum, or urine testing (ACOG & ASRM, 2012).

Finally, the guideline wrote that "there is no evidence that hormonal levels in saliva are biologically meaningful. In addition, whereas saliva is an ultrafiltrate of the blood and in theory should be amenable to testing for ''free'' (unbound) concentrations of hormones, salivary testing does not currently offer an accurate or precise method of hormone testing" (ACOG & ASRM, 2012). This guideline was reaffirmed in 2020.

The ACOG and ASRM published a clinical consensus stating that "although proponents claim that salivary testing can help tailor hormone therapy, salivary testing does not offer accurate or precise assessment of hormone levels. Steroid hormones mostly are bound to albumin, with less than five percent circulating in free form. Estrogen levels are extremely low in saliva, which make it methodologically challenging to measure. Progesterone is present in the saliva at higher levels, but circulating levels do not necessarily reflect the levels present in the tissue." Currently, there are no FDA-approved salivary or urinary tests for steroid hormone measurement (ACOG, 2023).

North American Menopausal Society (NAMS)

The NAMS addressed salivary hormone testing with regards to MHT, stating that salivary hormone testing is "inaccurate and unreliable." The NAMS further notes that the levels in serum, saliva, and tissue are "markedly different" and alludes to the FDA's statement that there is "no scientific basis for using saliva testing to adjust hormone levels" (NAMS, 2012).

The NAMS also addressed salivary hormone testing in the context of compounded HT (hormone therapy), which would include estradiol, estrone, and micronized progesterone (MP), but corroborates that salivary testing for HT is considered "unreliable because of differences in hormone pharmacokinetics and absorption, diurnal variation, and inter-individual and intraindividual variability" (NAMS, 2017).

Endocrine Society (ES)

The ES states that "salivary hormone assays are not standardized, do not have independent quality control programs, and lack an accepted reference range." The Society further mentions that there is no scientific evidence that a correlation exists between symptoms and salivary hormones. Assessment or monitoring of hormone therapy lacks evidence, and the American College of Obstetricians and Gynecologists, the North American Menopausal Society, and the Endocrine Society all recommend against salivary hormone testing to assess or monitor hormone levels because "they lack a rationale and therefore lead to unnecessary expense of treatment" (Santoro et al., 2016).

The ES also recommends a test of at least two LNSC measurements for diagnosis of Cushing Syndrome. If a patient has eucortisolism after a transsphenoidal selective adenomectomy (TSS), a measurement of late-night salivary or serum cortisol is recommended (Nieman, 2015; Nieman et al., 2008).

Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: http://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Salivary hormones may be measured by multiple tests. Additionally, many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDT's are not approved or cleared by the U. S.

Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
82530	Cortisol free
82533	Cortisol; total
82626	Dehydroepiandrosterone (dhea)
82627	Dehydroepiandrosterone
82670	Estradiol; total
82671	Estrogens; fractionated
82672	Estrogens; total
82677	Estriol
82679	Estrone
82681	Estradiol; free, direct measurement (eg, equilibrium dialysis)
84144	Progesterone
84402	Testosterone; free
84403	Testosterone; total
84410	Testosterone; bioavailable, direct measurement (eg, differential precipitation)
S3650	Saliva test, hormone level; during menopause

Procedure codes appearing in policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

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Revision History

Revision Date	Summary of Changes
03/06/2024	Reviewed and Updated: Updated the background, guidelines and recommendations, and evidence-based scientific references. Literature review did not necessitate any modifications to coverage criteria. The following edits were made for clarity and consistency: CC1, changed "the diagnosis of" to "individuals with signs and symptoms of" Cushing syndrome.
03/01/2023	Reviewed and Updated: Updated the background, guidelines and recommendations, and evidence-based scientific references. Literature review did not necessitate any modifications to coverage criteria. The following edits were made for clarity: All CC edited for clarity and consistency.
03/09/2022	Annual review: Updated background, guidelines, and evidence-based scientific references. Literature review did not necessitate any modification to coverage criteria.
03/03/2021	Annual review: Updated background, guidelines, and evidence-based scientific references. Literature review did not necessitate any modification to coverage criteria.
03/10/2020	Annual review: Updated background, guidelines, and evidence-based scientific references. Added in the statement regarding the lack of published literature for the previous E&I CC and changed E&I to DNMCC. Otherwise, literature review did not necessitate any additional modifications to the coverage criteria.
03/01/2019	Annual review: Updated definition, background, guidelines, and evidence-based scientific references. Reordered the CCs so that the MCC is posted first according to AHS protocol. Added conditions to the DNMCC and added cortisol to the DNMCC for those conditions. Revisions did not necessitate coding changes.
03/16/2018	Off-Cycle Review: Policy was reviewed to change the Annual Review Cycle. Literature review did not necessitate any modification to coverage criteria. No changes to coding.
09/28/2017	Annual review: Guidelines and Recommendations, Evidence-based Scientific References were updated. Literature review did not necessitate change in CC.
09/19/2016	Annual review completed. Literature review did not necessitate modification of coverage criteria. References updated.

09/18/2015	Initial presentation

Serum Biomarker Testing for Multiple Sclerosis and Related Neurologic Diseases

Policy Number: AHS – G2123 – Serum Biomarker Testing for Multiple Sclerosis and Related Neurologic Diseases	Prior Policy Name and Number, as applicable: AHS – G2123 – Serum Biomarker Tests for Multiple Sclerosis
Initial Presentation Date: 05/23/2016 Revision Date: February 1, 2025	

Policy Description

Multiple sclerosis (MS) is the most common immune-mediated inflammatory demyelinating disease of the central nervous system (CNS) and is defined by multifocal areas of demyelination with loss of oligodendrocytes and astroglial scarring. The most commonly present symptom is sensory disturbances, followed by weakness and visual disturbances. However, the disease has a highly variable pace and many atypical forms (Olek, 2022a). Besides MS, acute CNS demyelination also occurs in acute disseminated encephalomyelitis (ADEM), optic neuritis, transverse myelitis, and neuromyelitis optica (Lotze, 2022).

Neuromyelitis optica and neuromyelitis optica spectrum disorders (NMOSD) are inflammatory disorders of the CNS characterized by severe, immune-mediated demyelination and axonal damage predominantly targeting the optic nerves and spinal cord. Previously considered a subset of MS, this set of disorders is now recognized as its own clinical entity with its own unique immunologic features (Glisson, 2022).

Related Policies

Policy Number	Policy Title

N/A	

Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) For the diagnosis of multiple sclerosis (MS), cerebrospinal fluid (CSF) and serum oligoclonal band analysis **MEETS COVERAGE CRITERIA** in **any** of the following situations:
   a) For individuals with atypical clinical, laboratory, or imaging features.
   b) For individuals with an atypical, clinically isolated syndrome, including, but not limited to, primary progressive multiple sclerosis or relapsing-remitting course.
   c) For individuals belonging to a population in which MS is less common (e.g., children, older individuals).
   d) For individuals with insufficient clinical or imaging evidence for diagnosis.

2) In cases of suspected neuromyelitis optica spectrum disorders (NMOSD) or myelin oligodendrocyte glycoprotein-immunoglobulin G (MOG-IgG)-associated encephalomyelitis (MOG-EM), serum indirect fluorescence assay or fluorescence-activated cell sorting (FACS) assay of aquaporin-4-IgG (AQP4-IgG) and MOG-IgG **MEET COVERAGE CRITERIA** when **all** of the following conditions are met:
   a) The individual has monophasic or relapsing acute optic neuritis, myelitis, brainstem encephalitis, encephalitis, or any combination thereof;
   b) The individuals have radiological or electrophysiological findings compatible with central nervous system (CNS) demyelination;
   c) The individual has at least one of the following:
      i) Belongs to a higher risk population (e.g, pediatric).
      ii) Has an abnormal MRI depicting extensive optic nerve lesion, extensive spinal cord lesion or atrophy, or large confluent T2 brain lesions.
      iii) Has prominent papilledema/papillitis/optic disc swelling during acute optic neuritis.
      iv) Has neutrophilic CSF pleocytosis.
      v) Has a histopathology finding of primary demyelination with intralesional complement and IgG deposits or has a previous diagnosis of "pattern II MS".
      vi) Has simultaneous bilateral acute optic neuritis.
      vii) Has a severe visual deficit or blindness in one or both eyes during or after acute optic neuritis.
      viii) Has severe or frequent episodes of acute myelitis or brainstem encephalitis.
      ix) Has permanent sphincter and/or erectile disorder after myelitis.
      x) Has a previous diagnosis of acute disseminated encephalomyelitis (ADEM).

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

3) In all other situations, serum biomarker tests for multiple sclerosis **DO NOT MEET COVERAGE CRITERIA**.

4) ELISA, Western blot, immunohistochemistry, or any other serum assays to test for NMOSD or MOG-EM **DO NOT MEET COVERAGE CRITERIA**.

5) For the diagnosis of MS, NMOSD, or MOG-EM, all other cerebrospinal fluid (CSF) biomarker tests, including AQP4-IgG or MOG-IgG, **DO NOT MEET COVERAGE CRITERIA**.

Table of Terminology

Term	Definition
ADEM	Acute disseminated encephalomyelitis
AQP4Ab	Aquaporin-4 autoantibody
AQP4-IgG	Aquaporin-4-immunoglobulin G
AQP4-ON	Aquaporin-4 immunoglobulin G-Associated ON
BMI	Body mass index
CBA	Cell-Based immunofluorescence assay
CHI3L1	Chitinase3-like1
CIS	Clinically isolated syndrome
CLIA '88	Clinical Laboratory Improvement Amendments Of 1988
CMS	Centers For Medicare and Medicaid
CNS	Central nervous system
CPT	Current procedural terminology
CSF	Cerebrospinal fluid
EDSS	Expanded disability status scale
ELISA	Enzyme-linked immunosorbent immunoassay
FACS	Fluorescence-activated cell sorting
FDA	Food And Drug Administration
GCIPL	Ganglion cell + inner plexiform layer
GEL	Gadolinium-enhanced lesions
HCLA	High-contrast letter acuity
IPND	International Panel on MOG Encephalomyelitis
IVIG	Intravenous immunoglobulin treatment
LDTs	Laboratory-developed tests
miRNA	Micro ribonucleic acid
MOG	Myelin oligodendrocyte glycoprotein immunoglobulin G
MOG-EM	Myelin oligodendrocyte glycoprotein-immunoglobulin G-associated encephalomyelitis
MOG-IgG	Myelin oligodendrocyte glycoprotein-immunoglobulin G
MOG-ON	Myelin oligodendrocyte glycoprotein-immunoglobulin G-associated ON
MRI	Magnetic resonance imaging
mRNA	Messenger ribonucleic acid
MS	Multiple sclerosis
MS-ON	Multiple sclerosis-associated ON

NfL	Neurofilament light
NICE	National Institute for Health and Care Excellence
NMO	Neuromyelitis optica
NMOSD	Neuromyelitis optica spectrum disorders
OCBs	Oligoclonal immunoglobulin G Bands
ON	Optic neuritis
PPMS	Primary progressive multiple sclerosis
rON	Recurrent optic neuritis
RRMS	Relapsing-remitting multiple sclerosis
sNfL	Serum neurofilament light chain
SPMS	Secondary progressive multiple sclerosis

Scientific Background

In the United States, the 2023 estimated prevalence of multiple sclerosis (MS) is 288 per 100,000 individuals, totaling 913,925 persons with MS (Atlas of MS, 2023). The mean age of MS onset is 28 to 31 years of age with clinical disease usually becoming apparent between the ages of 15 to 45 years, though in rare instances, onset has been noted as early as the first years of life or as late as the seventh decade (Goodin, 2014). Prevalence of MS is highest in the 55- to 65- year age group (Wallin et al., 2019).

In most, but not all, cases, a patient presents with a clinically isolated syndrome (CIS) as the first single clinical event. This CIS preludes a clinically definite MS (Lublin et al., 2014). The pattern and course of MS is then further categorized into several clinical subtypes (Lublin et al., 2014): Relapsing-remitting MS (RRMS), secondary progressive MS (SPMS), and primary progressive MS (PPMS). RRMS is the most common type of disease course (85 to 90 percent of cases at onset (Weinshenker, 1994)) and is characterized by clearly defined relapses with full recovery, or with sequelae and residual deficit upon recovery. The transition from RRMS to SPMS usually occurs 10 to 20 years after disease onset (Eriksson et al., 2003). SPMS is characterized by an initial RRMS disease course followed by gradual worsening with or without occasional relapses, minor remissions, and plateaus. PPMS is characterized by progressive accumulation of disability from disease onset with occasional plateaus, temporary minor improvements, or acute relapses still consistent with the definition. A diagnosis of PPMS is made exclusively on patient history: there are no imaging or exam findings that distinguish PPMS from RRMS. PPMS represents about 10 percent of MS cases at disease onset (Koch et al., 2009; Olek, 2022a). Worsening of disability due to MS is highly variable. The impact of MS varies according to several measures, including severity of signs and symptoms, frequency of relapses, rate of worsening, and residual disability. Worsening of disability over time is a critical issue for MS patients (Olek, 2022a). Current treatments can delay the progression of the disease. However, this delay is only achievable if treatment starts at the beginning of the disease. Thus, it is essential that a proper diagnosis is made as early as possible, allowing for early treatment and as much delay as possible in symptom progression (Sapko et al., 2020).

Multiple sclerosis is primarily diagnosed clinically. The core requirement for the diagnosis is the demonstration of central nervous system lesion dissemination in time and space, based upon either clinical findings alone or a combination of clinical and MRI findings. The history and physical examination are most important for diagnostic purposes. MRI is the test of choice to support the clinical diagnosis of MS (Filippi & Rocca, 2011). The McDonald diagnostic criteria include specific MRI criteria for the demonstration of lesions dissemination in time and space; however, the McDonald criteria are not intended for distinguishing MS from other neurologic conditions (Brownlee et al., 2017). The sensitivity

and specificity of MRI for the diagnosis of MS varies widely in different studies. This variation is probably due to differences among the studies in MRI criteria and patient populations (Offenbacher et al., 1993; Schaffler et al., 2011). Using the 2010 McDonald criteria, the sensitivity and specificity were approximately 53 and 87 percent, respectively (Rovira et al., 2009). In the first studies applying the 2017 criteria (Hyun et al., 2018), the sensitivity is higher (83.6%), but the specificity is lower (85%).

Qualitative assessment of cerebrospinal fluid (CSF) for oligoclonal IgG bands (OCBs) using isoelectric focusing can be an important diagnostic tool when determining a diagnosis of MS. Elevation of the CSF immunoglobulin level relative to other protein components is a common finding in patients with MS and suggests intrathecal synthesis. The immunoglobulin increase is predominantly IgG, although the synthesis of IgM and IgA is also increased (Olek, 2022a). A positive finding is defined by "finding of either oligoclonal bands different from any such bands in serum, or by an increased IgG index" and can be measured by features such as percentage of total protein or total albumin. Up to 95% of clinically definite MS cases will have these oligoclonal bands (Olek, 2022b).

The 2017 McDonald criteria allows for the presence of CSF oligoclonal bands to substitute for the diagnostic requirement of fulfilling dissemination in time. However, Thompson notes that "currently, no laboratory test in isolation confirms the diagnosis of multiple sclerosis" (Thompson et al., 2018). Luzzio (2023) also note that in a review of four guidelines from the Consortium of Multiple Sclerosis Centers, the European Academy of Neurology, and the Magnetic Resonance Imaging in MS Network, MRI is the "imaging procedure of choice for confirming MS and monitoring disease progression in the brain and spinal cord" (Luzzio, 2023).

Neuromyelitis optica spectrum disorders (NMOSD, also known as Devic disease or neuromyelitis optica, NMO) are a range of conditions that are characterized by symptoms similar to MS; namely demyelination and axonal damage to structures of the central nervous system, such as the spinal cord. Previously, NMOSD were considered a subset of MS; however, now NMOSD and NMO are recognized as having distinct features, specifically the presence of a NMOSD/NMO-specific antibody that binds aquaporin-4 (AQP4), setting these apart from relapsing-remitting MS. AQP4 is a water channel protein primarily located in the spinal cord gray matter. NMO-IgG (or anti-AQP4) is involved in the pathogenesis of NMOSD/NMO. This antibody selectively binds AQP4, differing from MS in that the loss of AQP4 expression is unrelated to the stage of demyelination. The presence of this antibody is incorporated into the current diagnostic criteria for NMOSD and can differentiate MS cases from NMOSD cases (Glisson, 2022).

Several novel MS-related prognostic biomarkers are being investigated for clinical use. Serum neurofilament light chain (sNfl) has been implicated as a potential marker; however, it is clinically difficult to evaluate individual patients with NfL because of confounding variables; NfL can indicate neuroinflammation (rather than neurodegeneration). Other biomarkers of axonal damage, neuronal damage, glial dysfunction, demyelination, and inflammation are beset by similar issues as well as limited by conflicting results from studies. According to Yang et al. (2022), future practice could benefit from integrating a diverse set of biomarkers (a combination of proteins, transcriptomics, immune cells, extracellular vessels, metabolites, and the microbiome). Scientists could use cutting-edge bioinformatics to identify and predict disease progression. Other promising technologies may aid in the discovery of new biomarkers such as proteomics, metabolomics, and sc-RNA seq (Yang et al., 2022).

Clinical Utility and Validity

There is a strong unmet clinical need for objective body fluid biomarkers to assist early diagnosis and estimate long-term prognosis, monitor treatment response, and predict potential adverse effects in MS. Currently, no biomarkers of MS have been validated; however, many are under consideration: microRNA (miRNA), messenger RNA (mRNA), lipids, autoantibodies, metabolites, and proteins all have been reported to have potential as possible biomarkers (Comabella & Montalban, 2014; Comabella et al., 2016; El Ayoubi & Khoury, 2017; Lim et al., 2017; Raphael et al., 2015; Teunissen et al., 2015).

Fryer et al. (2014) compared three assays for measuring aquaporin-4 IgG: ELISA, fixed cell-based fluorescence (CBA), and live cell-based fluorescence (FACS, M1 and M23 versions). Four groups of patients were measured with these assays. In Group one (n = 388), FACS was optimal, with the highest area under the curve. In Group two, FACS identified the highest percentage of neuromyelitis optica spectrum disorders, identifying 23 (M1) and 24 (M23) of 30 patients. In Group three, all four assays identified true negatives at an approximate 85% success rate (5 of 31 positives). In Group four, all four assays identified true positives in 40 of 41 samples. The authors noted that "aquaporin-4-transfected CBAs, particularly M1-FACS, perform optimally in aiding NMOSD serologic diagnosis" (Fryer et al., 2014).

Jitprapaikulsan et al. (2018) evaluated the prognostic value of aquaporin-4 IgG and myelin oligodendrocyte glycoprotein IgG (MOG) in patients with recurrent optic neuritis (rON). The study included 246 and autoantibodies were detected in 32% of these patients (aquaporin-4 in 19%, MOG in 13%), 186 patients had rON only and 60 patients had "additional inflammatory demyelinating attacks" (rON plus). Of the 186 rON only patients, 27 were positive for MOG, 24 were positive for aquaporin-4, and 110 were negative for both. In the rON plus group, 23 were positive for aquaporin-4, 4 were positive for MOG, and 11 were negative for both. The authors noted that five years after optic neuritis onset, 59% of aquaporin-4 positive patients and 12% of MOG positive patients were estimated to have "severe visual loss". The authors concluded that "aquaporin-4 IgG seropositivity predicts a worse visual outcome than MOG IgG1 seropositivity, double seronegativity, or MS diagnosis. Myelin oligodendrocyte glycoprotein IgG1 is associated with a greater relapse rate but better visual outcomes" (Jitprapaikulsan et al., 2018).

Sotirchos et al. (2019) compared 31 healthy controls with individuals with one of three types of optic neuritis (ON): 48 individuals with aquaporin-4 IgG-associated ON (AQP4-ON), 16 individuals with myelin oligodendrocyte glycoprotein-IgG-associated ON (MOG-ON), and 40 individuals with MS-associated ON (MS-ON). The authors note, "AQP4-ON eyes exhibited worse high-contrast letter acuity (HCLA) compared to MOG-ON (-22.3 ± 3.9 letters; $p < 0.001$) and MS-ON eyes (-21.7 ± 4.0 letters; $p < 0.001$). Macular ganglion cell + inner plexiform layer (GCIPL) thickness was lower, as compared to MS-ON, in AQP4-ON (-9.1 ± 2.0 µm; $p < 0.001$) and MOG-ON (-7.6 ± 2.2 µm; $p = 0.001$) eyes. Lower GCIPL thickness was associated with worse HCLA in AQP4-ON (-16.5 ± 1.5 letters per 10 µm decrease; $p < 0.001$) and MS-ON eyes (-8.5 ± 2.3 letters per 10 µm decrease; $p < 0.001$), but not in MOG-ON eyes (-5.2 ± 3.8 letters per 10 µm decrease; $p = 0.17$), and these relationships differed between the AQP4-ON and other ON groups ($p < 0.01$ for interaction)." These data indicate that AQP4-IgG seropositivity suggests worse visual outcomes than those occurring after MOG-ON or even MS-ON (Sotirchos et al., 2019).

Cantó et al. (2019) evaluated neurofilament light chain's (NfL) ability to "serve as a reliable biomarker of disease worsening for patients with multiple sclerosis (MS)." The study included 607 patients with MS; patients were assessed over a period of 12 years. Serum NfL was measured, and disability progression was the primary clinical outcome (defined as "clinically significant worsening on the Expanded Disability Status Scale (EDSS) score and brain fraction atrophy"). Baseline measurements of NfL showed significant association with EDSS score, MS subtype, and treatment status. Worsening EDSS scores and changes of NfL levels over time were found to be correlated. The baseline NfL measurement was also found to be

associated with approximately 11.6% of brain fraction atrophy over 10 years, increasing to 18% after multivariable analysis. Furthermore, active treatment was associated with declining levels of NfL, with "high-potency treatments" associated with the greatest decrease out of all of the treatments assessed. Overall, the authors concluded that they had confirmed a significant association of serum NfL with clinical outcomes of MS. However, they also acknowledged that "further prospective studies are necessary to assess the assay's utility for decision-making in individual patients" (Cantó et al., 2019).

Gil-Perotin et al. (2019) evaluated the combined biomarker profile of NfL and chitinase3-like1 (CHI3L1) and its ability to provide prognostic information for patients with MS. 157 MS patients were included, with 99 RRMS patients, 35 SPMS patients, and 23 PPMS patients. Disease activity was defined by "clinical relapse and/or gadolinium-enhanced lesions (GEL) in MRI within 90 days from CSF collection." Levels of both biomarkers were found to be higher in MS patients compared to non-MS patients. Elevated NfL was associated with clinical relapse and GEL in RRMS and SPMS patients and high CHI3L1 levels were characteristic of progressive disease. The authors also found the combined profile useful for differentiating between MS subtypes, with high NfL and low CHI3L1 often indicating a RRMS stage. They found that elevation of both biomarkers indicates disease progression. Overall, the authors concluded these biomarkers were useful for disease activity and progression and that the biomarker profile can discriminate between MS subtypes (Gil-Perotin et al., 2019).

Martin et al. (2019) performed a meta-analysis to evaluate the CSF levels of NfL to determine "whether, and to what degree, CSF NfL levels differentiate MS from controls, or the subtypes or stages of MS from each other". The authors identified 14 articles for inclusion in their meta-analysis. NfL levels were higher in MS patients (746) than controls (435) (mean of 1965.8 ng/L in MS patients compared to 578.3 ng/L in healthy controls). Mean NfL levels were found to be higher in 176 patients with relapsing disease (mean = 2124.8ng/L) compared to 92 patients with progressive disease (mean = 1121.4ng/L). The authors also found that patients with relapsing disease (138 in this cohort) had approximately double the levels of CSF NfL compared to patients in remission (268), with an average of 3080.6ng/L in the relapsing cohort compared to 1541.7ng/L in the remission cohort. Overall, the authors concluded that CSF NfL correlates with MS activity throughout the course of disease, that relapse was strongly associated with elevated CSF NfL levels, and that CSF NfL may be useful as a measure of activity (Martin et al., 2019).

Simonsen et al. (2020) performed a retrospective study investigating if analysis of IgG index could safely predict oligoclonal band (OCB) findings. A total of 1295 MS patients were included, with 93.8% of them positive for OCBs. Of 842 MS patients with known IgG status and known OCB status, 93.3% were oligoclonal band positive and 76.7% were found to have an elevated IgG profile. The authors found the positive predictive value of elevated IgG based on positive OCBs to be 99.4%, and the negative predictive value of normal IgG based on negative OCBs to be 26.5%. The authors concluded that an IgG index of >0.7 has a positive predictive value of >99% for OCBs (Simonsen et al., 2020).

Benkert et al. (2022) conducted a retrospective modelling and validation study aiming to assess the ability of serum neurofilament light chain (sNfL) to identify people at risk of future MS. The authors used a reference database to determine reference values of sNfL corrected for age and body mass index (BMI). The study included a control group (no history of CNS disease) and MS patients. In the control group, sNfL concentrations increased exponentially with age; the rate of increase rose after the age of 50. In MS patients, "sNfL percentiles and Z scores indicated a gradually increased risk for future acute (eg, relapse and lesion formation) and chronic (disability worsening) disease activity." The authors collected data before and after MS treatment and found that sNfL Z score values decreased to the level of the control group with monoclonal antibodies, and, to a lesser extent, with oral therapies. sNfL Z scores did not decrease with platform compounds such as interferons and glatiramer acetate. The

authors conclude that "use of sNfL percentiles and Z scores allows for identification of individual people with multiple sclerosis at risk for a detrimental disease course and suboptimal therapy response beyond clinical and MRI measures, specifically in people with disease activity-free status" (Benkert et al., 2022).

Guidelines and Recommendations

International Advisory Committee on Clinical Trials in Multiple Sclerosis

In 2014, the International Advisory Committee on Clinical Trials in Multiple Sclerosis, jointly sponsored by the U.S. National Multiple Sclerosis Society, the European Committee for Treatment and Research in Multiple Sclerosis, and the MS Phenotype Group, re-examined MS phenotypes, exploring clinical, imaging, and biomarker advances through working groups and literature searches. The committee concluded that "To date, there are no clear clinical, imaging, immunologic or pathologic criteria to determine the transition point when RRMS [relapse-remitting MS] converts to SPMS [secondary progressive MS]; the transition is usually gradual. This has limited our ability to study the imaging and biomarker characteristics that may distinguish this course" (Lublin et al., 2014). In 2020, the committee updated this policy for clarity, summarizing with "the committee urges clinicians, investigators, and regulators to consistently and fully use the 2013 phenotype characterizations by (1) using the full definition of activity, that is, the occurrence of a relapse or new activity on an MRI scan (a gadolinium-enhancing lesion or a new/unequivocally enlarging T2 lesion); (2) framing activity and progression in time; and (3) using the terms worsening and progressing or disease progression more precisely when describing MS course"(Lublin et al., 2020).

The International Panel on Diagnosis of Multiple Sclerosis

The Panel reviewed the 2010 McDonald criteria and recommended: "In a patient with a typical clinically isolated syndrome and fulfilment of clinical or MRI criteria for dissemination in space and no better explanation for the clinical presentation, demonstration of CSF-specific oligoclonal bands in the absence of other CSF findings atypical of multiple sclerosis allows a diagnosis of this disease to be made." The Panel goes on to state that "CSF oligoclonal bands are an independent predictor of the risk of a second attack when controlling for demographic, clinical, treatment, and MRI variables" and that in the absence of atypical CSF findings, demonstration of these CSF OCBs can allow for a diagnosis of MS to be made. The Panel remarks that inclusion of this CSF criterion can substitute for the traditional "dissemination in time" criterion, but that no laboratory test in isolation can confirm an MS diagnosis (Thompson et al., 2018).

Cerebrospinal fluid examination is "strongly recommended" in some circumstances for MS diagnosis, and the Panel remarks that the threshold for additional testing should be low. Those circumstances are as follows:

- "when clinical and brain MRI evidence supporting a diagnosis of multiple sclerosis is insufficient, particularly if initiation of long-term disease-modifying therapies are being considered"
- "when there is a presentation other than a typical clinically isolated syndrome, including patients with a progressive course at onset (primary progressive multiple sclerosis)"
- "when there are clinical, imaging, or laboratory features atypical of MS"
- "in populations in which diagnosing MS is less common (for example, children, older individuals, or non-Caucasians)."

The Panel does emphasize that it is essential for CSF to be paired with another serum sample when analyzed to demonstrate that the OCBs are unique to the CSF (Thompson et al., 2018).

The treatments for these similar conditions (MS and NMOSD) differ, as some MS treatments (interferon beta, fingolimod, and natalizumab) can exacerbate NMOSDs. Therefore, the Panel recommended that "NMOSDs should be considered in any patient being evaluated for multiple sclerosis". The Panel notes that aquaporin-4 serological testing "generally differentiates" NMOSD from MS (Thompson et al., 2018). Serological testing for AQP4 and for MOG should be done in all patients with features suggesting NMOSDs (severe brainstem involvement, bilateral optic neuritis, longitudinally extensive spinal cord lesions, large cerebral lesions, or a normal brain MRI or findings not fulfilling dissemination in space [DIS]), and considered in groups at higher risk of NMOSDs (African American, Asian, Latin American, and pediatric populations) (Thompson et al., 2018).

International Panel on MOG Encephalomyelitis (IPND)

Human myelin oligodendrocyte glycoprotein (MOG-IgG)-associated encephalomyelitis (MOG-EM) is considered a unique disease from MS and other NMOSD, but MOG-EM has often been misdiagnosed as MS in the past. In 2018, an international panel released their recommendations concerning diagnosis and antibody testing. They state their purpose with the following: "To lessen the hazard of overdiagnosing MOG-EM, which may lead to inappropriate treatment, more selective criteria for MOG-IgG testing are urgently needed. In this paper, we propose indications for MOG-IgG testing based on expert consensus. In addition, we give a list of conditions atypical for MOG-EM ("red flags") that should prompt physicians to challenge a positive MOG-IgG test result. Finally, we provide recommendations regarding assay methodology, specimen sampling and data interpretation" (Jarius et al., 2018).

They list the following recommendations:

- Assay: Indirect fluorescence assays, including fluorescence-activated cell sorting (FACS) that targets full-length human MOG (IgG-specific), are the gold standards. The use of either IgM or IgA antibodies are less specific and can result in both false-negative results due to high-affinity IgG displacing IgM and false-positive results due to cross-reactivity with rheumatoid factors.
- Immunohistochemistry is NOT recommended because it is "less sensitive than cell-based assays, limited data available on specificity, [and] sensitivity depends on tissue donor species."
- Peptide-based ELISA and Western blot are NOT recommended because they are "insufficiently specific, obsolete."
- Biomaterial: Serum is the recommended specimen of choice. CSF is "not usually required" because "MOG-IgG is produced mostly extrathecally, resulting in lower CSF than serum titers."
- Timing of testing: Serum concentration of MOG-IgG is highest during an acute attack and/or while not receiving immunosuppressive treatment. MOG-IgG concentration may decrease during remission. "If MOG-IgG test is negative but MOG-EM is still suspected, re-testing during acute attacks, during treatment-free intervals, or 1-3 months after plasma exchange (or IVIG [intravenous immunoglobulin treatment]) is recommended."
- "Given the very low pre-test probability, we recommend against general MOG-IgG testing in patients with a progressive disease course."
- "In practice, many patients diagnosed with AQP4-IgG-negative NMOSD according to the IPND 2015 criteria will meet also the criteria for MOG-IgG testing…and should thus be tested. However, MOG-IgG testing should not be restricted to patients with AQP4-IgG-negative NMOSD" (Jarius et al., 2018).

The table below outlines the recommendation on the criteria required for testing:

Table 1 Recommended indications for MOG-IgG testing in patients presenting with acute CNS demyelination of putative autoimmune etiology

1. Monophasic or relapsing acute optic neuritis, myelitis, brainstem encephalitis, encephalitis, or any combination thereof,
and
2. radiological or, only in patients with a history of optic neuritis, electrophysiological (VEP) findings compatible with CNS demyelination,
and
3. at least one of the following findings:

MRI
- a. Longitudinally extensive spinal cord lesion (≥3 VS, contiguous) on MRI (so-called LETM)[a,b]
- b. Longitudinally extensive spinal cord atrophy (≥3 VS, contiguous) on MRI in patients with a history compatible with acute myelitis[a]
- c. Conus medullaris lesions, especially if present at onset[c]
- d. Longitudinally extensive optic nerve lesion (e.g., >1/2 of the length of the pre-chiasmal optic nerve, T2 or T1/Gd)[d]
- e. Perioptic Gd enhancement during acute ON[e]
- f. Normal supratentorial MRI in patients with acute ON, myelitis and/or brainstem encephalitis
- g. Brain MRI abnormal but no lesion adjacent to a lateral ventricle that is ovoid/round or associated with an inferior temporal lobe lesion and no Dawson's finger-type or juxtacortical U fiber lesion (Matthews-Jurynczyk criteria[f])
- h. Large, confluent T2 brain lesions suggestive of ADEM

Fundoscopy
- i. Prominent papilledema/papillitis/optic disc swelling during acute ON

CSF
- j. Neutrophilic CSF pleocytosis[g]or CSF WCC > 50/μl[h]
- k. No CSF-restricted OCB as detected by IEF at first or any follow-up examination[i] (applies to continental European patients only)

Histopathology
- l. Primary demyelination with intralesional complement and IgG deposits
- m. Previous diagnosis of "pattern II MS" [j]

Clinical findings
- n. Simultaneous bilateral acute ON
- o. Unusually high ON frequency or disease mainly characterized by recurrent ON
- p. Particularly severe visual deficit/blindness in one or both eyes during or after acute ON
- q. Particularly severe or frequent episodes of acute myelitis or brainstem encephalitis
- r. Permanent sphincter and/or erectile disorder after myelitis
- s. Patients diagnosed with "ADEM", "recurrent ADEM", "multiphasic ADEM" or "ADEM-ON"
- t. Acute respiratory insufficiency, disturbance of consciousness, behavioral changes, or epileptic seizures (radiological signs of demyelination required)
- u. Disease started within 4 days to ~ 4 weeks after vaccination
- v. Otherwise unexplained intractable nausea and vomiting or intractable hiccups (compatible with area postrema syndrome)[a]
- w. Co-existing teratoma or NMDAR encephalitis (low evidence[k])

Treatment response
- x. Frequent flare-ups after IVMP, or steroid-dependent symptoms[l] (including CRION)
- y. Clear increase in relapse rate following treatment with IFN-beta or natalizumab in patients diagnosed with MS (low evidence)

Note that these recommendations are primarily intended for use in adults and adolescents. Indications for MOG-IgG testing in young children need not to be as rigorous

International Panel on NMOSD

The International Panel on NMOSD recommends "testing with cell-based serum assays (microscopy or flow cytometry-based detection) whenever possible because they optimize autoantibody detection (mean sensitivity 76.7% in a pooled analysis; 0.1% false-positive rate in a MS clinic cohort)." They state that ELISA and indirect immunofluorescence assays have lower sensitivity and "strongly" recommend "interpretative caution if such assays are used and when low-titer positive ELISA results are detected in individuals who present with NMOSD clinical symptoms less commonly associated with AQP4-IgG (e.g., presentations other than recurrent optic neuritis, myelitis with LETM, or area postrema syndrome) or in situations where clinical evidence suggests a viable alternate diagnosis. Confirmatory testing is recommended, ideally using 1 or more different AQP4-IgG assay techniques. Cell-based assay has the best current sensitivity and specificity and samples may need to be referred to a specialized laboratory." The table below outlines the NMOSD diagnostic criteria for adult patients (Wingerchuk et al., 2015).

Table 1 NMOSD diagnostic criteria for adult patients

Diagnostic criteria for NMOSD with AQP4-IgG

1. At least 1 core clinical characteristic
2. Positive test for AQP4-IgG using best available detection method (cell-based assay strongly recommended)
3. Exclusion of alternative diagnoses[a]

Diagnostic criteria for NMOSD without AQP4-IgG or NMOSD with unknown AQP4-IgG status

1. At least 2 core clinical characteristics occurring as a result of one or more clinical attacks and meeting all of the following requirements:
   a. At least 1 core clinical characteristic must be optic neuritis, acute myelitis with LETM, or area postrema syndrome
   b. Dissemination in space (2 or more different core clinical characteristics)
   c. Fulfillment of additional MRI requirements, as applicable
2. Negative tests for AQP4-IgG using best available detection method, or testing unavailable
3. Exclusion of alternative diagnoses[a]

Core clinical characteristics

1. Optic neuritis
2. Acute myelitis
3. Area postrema syndrome: episode of otherwise unexplained hiccups or nausea and vomiting
4. Acute brainstem syndrome
5. Symptomatic narcolepsy or acute diencephalic clinical syndrome with NMOSD-typical diencephalic MRI lesions (figure 3)
6. Symptomatic cerebral syndrome with NMOSD-typical brain lesions (figure 3)

National Institute for Health and Care Excellence (NICE)

The 2022 NICE guidelines on MS in adults recommends diagnosing MS using a "combination of history, examination, MRI and laboratory findings, and by following the 2017 revised McDonald criteria" and notes that this should include "looking for cerebrospinal fluid-specific oligoclonal bands if there is no clinical or radiological evidence of lesions developing at different times" (NICE, 2022).

Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

In 2016, the FDA approved the KRONUS Aquaporin-4 Autoantibody (AQP4Ab) ELISA Assay. The indication for use is as follows: "The KRONUS Aquaporin-4 Autoantibody (AQP4Ab) ELISA Assay is for the semi-quantitative determination of autoantibodies to Aquaporin-4 in human serum. The KRONUS Aquaporin-4 Autoantibody (AQP4Ab) ELISA Assay may be useful as an aid in the diagnosis of Neuromyelitis Optica (NMO) and Neuromyelitis Optica Spectrum Disorders (NMOSD). The KRONUS

Aquaporin-4 Autoantibody (AQP4Ab) ELISA Assay is not to be used alone and is to be used in conjunction with other clinical, laboratory, and radiological (e.g. MRI) findings" (FDA, 2016).

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
83520	Immunoassay for analyte other than infectious agent antibody or infectious agent antigen; quantitative, not otherwise specified
83916	Oligoclonal immune (oligoclonal bands)
84182	Protein; Western Blot, with interpretation and report, blood or other body fluid, immunological probe for band identification, each
86051	Aquaporin-4 (neuromyelitis optica [NMO]) antibody; enzyme-linked immunosorbent immunoassay (ELISA)
86052	Aquaporin-4 (neuromyelitis optica [NMO]) antibody; cell-based immunofluorescence assay (CBA), each
86053	Aquaporin-4 (neuromyelitis optica [NMO]) antibody; flow cytometry (ie, fluorescence-activated cell sorting [FACS]), each
86362	Myelin oligodendrocyte glycoprotein (MOG-IgG1) antibody; cell-based immunofluorescence assay (CBA), each
86363	Myelin oligodendrocyte glycoprotein (MOG-IgG1) antibody; flow cytometry (ie, fluorescence-activated cell sorting [FACS]), each
88341	Immunohistochemistry or immunocytochemistry, per specimen; each additional single antibody stain procedure (List separately in addition to code for primary procedure)
88342	Immunohistochemistry or immunocytochemistry, per specimen; initial single antibody stain procedure

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

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Revision History

Revision Date	Summary of Changes
09/06/2023	Reviewed and Updated: Updated the background, guidelines and recommendations, and evidence-based scientific references. Literature review did not necessitate any modifications to coverage criteria. The following edits were made for clarity: ":" added at the end of the primary paragraph for CC1 Defined central nervous system (CNS) in CC2.b. Removed ethnicity as an example of a high risk population in CC1.c. and CC2.c.i., as other risk populations are already listed.

Serum Testing for Evidence of Mild Traumatic Brain Injury

Policy Number: AHS – G2151 – Serum Testing for Evidence of Mild Traumatic Brain Injury	Prior Policy Name and Number, as applicable:
Initial Presentation Date: 4/19/2018 Revision Date: February 1, 2025	

Policy Description

Traumatic brain injury (TBI) is characterized by pathologic injuries to the brain resulting from external forces or trauma. A broad range of sequela of varying clinical severity include focal contusions and hematomas, diffuse axonal injury, cerebral edema and swelling, and a cascade of molecular injury mechanisms (Williamson & Rajajee, 2021).

Concussion refers to the trauma-induced alteration in mental status, which may or may not involve loss of consciousness, after a mild TBI (Evans & Whitlow, 2022). Measurement of blood and other fluid biomarkers has been proposed as a way of evaluating mild traumatic brain injury.

Related Policies

Policy Number	Policy Title
	Not applicable

Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

1) For the evaluation of mild traumatic brain injury, measurement of concussion markers (e.g., S100B, GFAP, and UCH-L1) in the blood, saliva, and/or cerebrospinal fluid (CSF), including proprietary biomarker panels (e.g., i-STAT TBI Plasma, Alinity® i TBI), **DOES NOT MEET COVERAGE CRITERIA.**

Table of Terminology

Term	Definition
AAFP	American Academy of Family Physicians
AAOS	American Academy of Orthopedic Surgeons
AAP	American Academy of Pediatrics
ACEP	American College of Emergency Physicians
ACSM	American College of Sports Medicine
AMPAR	α-Amino-3-Hydroxy-5- Methyl-4-Isoxazolepropionic Acid Receptor
AMSSM	American Medical Society for Sports Medicine
AOASM	American Osteopathic Academy of Sports Medicine
AOSSM	American Orthopedic Society for Sports Medicine
AUC	Areas under the curve
BTI™	Brain trauma indicator
CDC	Centers for Disease Control
CISG	Concussion In Sport Group
CLIA '88	Clinical Laboratory Improvement Amendments Of 1988
CMS	Centers for Medicare and Medicaid
CNS	Central nervous system
CPT	Current procedural terminology
CSF	Cerebrospinal fluid
CT	Computed tomography
EEG	Electroencephalogram
ELISA	Enzyme-Linked Immunosorbent Assay
FDA	Food and Drug Administration
GFAP	Glial fibrillary acidic protein
GFAP-BDP	Glial fibrillary acidic protein breakdown products
GOS	Glasgow outcome score
HCPCS	Healthcare Common Procedure Coding System
JAMA	Journal of the American Medical Association
LDTs	Laboratory-developed tests
MMP-9	Matrix metalloproteinase 9
MMWR	Morbidity and Mortality Weekly Report
MRI	Magnetic resonance imaging
mTBI	Mild traumatic brain injury
NICE	National Institute of Care and Excellence
NSE	Neuron specific enolase
ONF	Ontario Neurotrauma Foundation
S100B	S100 calcium-binding protein b

sTBI	Severe traumatic brain injury
TBI	Traumatic brain injury
UCH-L1	Ubiquitin c-terminal hydrolase-l1
VA/DoD	Veterans Administration / Department of Defense

Scientific Background

Traumatic brain injury (TBI) is a fairly common injury, with an incidence of 1.11 million and a prevalence of 2.35 million in the US in 2016 (Evans & Whitlow, 2022). According to the CDC, there were over 64,000 TBI-related deaths in the United States in 2020 (CDC, 2023). Although approximately 75% of TBIs are mild, TBI can adversely affect a person's quality of life in numerous ways, including cognitive functioning, emotional functioning, and physical effects (CDC, 2015; Wright et al., 2013). As many as 1 in 5 TBI patients have symptoms persisting past 1 month (Silverberg et al., 2020).

Accurate diagnosis of TBI is critical to effective management and intervention but can be challenging due to the nonspecific and variable presentation (Mondello et al., 2017). Tools available to objectively diagnose injury and prognosticate recovery are limited (Mannix et al., 2014). Clinical assessment usually includes a neurological exam, followed by a computed tomography (CT) scan of the head to detect brain tissue damage that may require treatment (FDA, 2018). However, as most patients with mild TBI do not have detectable intracranial lesions, like epidural hematomas, on a CT scan (Evans & Whitlow, 2022), this assessment relies heavily on nonspecific symptoms that can vary widely and ignores the mechanistic heterogeneity of TBI (Williamson & Rajajee, 2021).

Brain damage in TBIs is initially caused by external mechanical forces being transferred to intracranial contents, generating shearing and strain forces which stretch and damage axons, and can result in contusions, hematomas, cerebral edema and swelling. Common mechanisms include direct impact, rapid acceleration/deceleration, penetrating injury, and blast waves. However, the pathophysiology of TBI is now understood to include not only the acute event, but also the resulting cascade of molecular injury mechanisms that are initiated at the time of initial trauma and continue for hours or days (Williamson & Rajajee, 2021). The pathophysiology of even mild TBI is complex and may include both focal and diffuse injury patterns. Neuropathological changes found after mild TBI indicate mild multifocal axonal injury, including altered circuit dysfunction and traumatic axonal injury (Truettner et al., 2018).

Cell death and the initiation of local metabolic and inflammatory processes resulting from TBI results in the release of a number of inflammatory mediators and damage-associated molecules that are able to cross a dysfunctional blood-brain barrier (Di Battista et al., 2015) or enter the circulation through the glymphatic pathway (Plog et al., 2015). Neurobiochemical marker levels in blood after TBI may reflect structural changes detected by neuroimaging (Mondello et al., 2017). Simpler, sensitive, and specific tests that provide early, quantitative information about the extent of brain tissue damage, identifying and stratifying TBI, would allow rapid and tailored diagnosis of TBI, while minimizing the time, risk, and cost associated with current standards (McMahon et al., 2015). No single ideal TBI biomarker exists (Halford et al., 2017). However, brain-specific markers of neuronal, glial, and axonal damage, identified in the peripheral blood, have shown potential clinical utility as diagnostic, prognostic, and monitoring adjuncts and have been investigated both individually and in combination (Di Battista et al., 2015; Mondello, Jeromin, et al., 2012). Acute-phase biomarkers, including S100 calcium-binding protein B (S100B), glial fibrillary acidic protein (GFAP), and ubiquitin C-terminal hydrolase-L1 (UCH-L1), have shown potential for use in initial screening of patients presenting with head trauma, the vast majority of whom will have normal brain CT findings (Evans & Whitlow, 2022; Maas et al., 2017).

However, recent reviews have noted concerns about a lack of specificity and unresolved issues with the use of mTBI blood biomarkers. While researchers note "impressive levels of sensitivity," they simultaneously acknowledge that correlations between blood biomarker levels and mTBI severity have been "disappointing to date." In particular, they state that it remains inconclusive whether biomarkers can predict recovery time, post-concussion syndrome, and/or return to sports activities (Hier et al., 2021).

S100 calcium-binding protein B (S100B)

S100B belongs to the calcium binding EF-hand protein group, and it has been associated with cytoskeleton structure, Ca^{2+} homeostasis, cell proliferation, protein phosphorylation and degradation (Chmielewska et al., 2018; Strathmann et al., 2014). S100B is expressed in the cytoplasm and the nucleus of astrocytes and is present in the bloodstream when the blood brain barrier is disrupted. Several studies indicate that S100B measurement, either acutely or at several time points, can distinguish injured from non-injured patient (Strathmann et al., 2014) and guidelines intended to reduce the need for CT scan using S100B levels in the blood for the initial management of mild TBI have been published (Ingebrigtsen et al., 2000). These guidelines were recently validated in a large multicenter study where S100B was found to have a sensitivity of 97% and a specificity of 34% for the identification of intracranial hemorrhages confirmed by CT scans. The authors estimated CT scans would have been reduced by 32% with application of these guidelines (Unden et al., 2015). However, other investigators have failed to detect associations between S100B with CT abnormalities (Piazza et al., 2007). Additionally, it has limited utility in multiple trauma settings as it is not brain-specific. S100B can be found in non-neural cells, such as adipocytes, chondrocytes, and melanocytes (Chmielewska et al., 2018; Papa et al., 2014), and its levels are also elevated in trauma, specifically orthopedic, without head injury (Anderson et al., 2001; Wang et al., 2018). However, recent data highlight the inclusion of S100B in sets of markers that in combination could contribute to better diagnosis, monitoring, and treatment of CNS conditions (Chmielewska et al., 2018).

Glial Fibrillary Acidic Protein (GFAP)

GFAP is a filament protein that maintains cell shape and structure, coordinates cells' mobility and contributes to the transduction of molecular signals in astrocytes. It is released upon cellular disintegration and degradation of the astrocyte. Concentration of serum GFAP increases after neural trauma and TBI, either as the intact protein or as breakdown products (Chmielewska et al., 2018; Wang et al., 2018). GFAP measurements have provided promising data on injury pathway indication, focal versus diffuse injuries, and prediction of morbidity and mortality (Strathmann et al., 2014). GFAP level was increased in patients with CT-positive scans for intracranial lesions compared to CT-negative scans after mild TBI (Lei et al., 2015). Sensitivities have been reported between 67% and 100% while the specificities ranged from 0% and 89% (Mondello et al., 2017).

McMahon et al (2015) performed a multicenter trial to evaluate GFAP and its breakdown product GFAP-BDP in the diagnosis of intracranial injury. They found that "GFAP-BDP demonstrated very good predictive ability (area under the curve=0.87) and demonstrated significant discrimination of injury severity (odds ratio, 1.45; 95% confidence interval, 1.29-1.64)". The authors concluded that "use of GFAP-BDP yielded a net benefit above clinical screening alone and a net reduction in unnecessary scans by 12-30%" (McMahon et al., 2015).

Ubiquitin C-terminal Hydrolase-L1 protein (UCH-L1)

UCH-L1 is a cytoplasmic enzyme, highly enriched and specifically expressed in neurons, involved in the ubiquitinoylation of abnormal proteins destined for proteasomal degradation (Halford et al., 2017). It is also an important element of axonal transport and, by a strong interaction with cytoskeleton proteins, plays an important role in the axon's integrity (Chmielewska et al., 2018). UCH-L1 has been shown to increase after TBI in serum and CSF as well as correlate with TBI severity and abnormal CT findings (Diaz-Arrastia et al., 2014; Wang et al., 2018). UCH-L1 has also been shown to be significantly elevated in serum among athletes after concussions (Wang et al., 2018). High prognostic value of poor outcome was found at both 3-months (Diaz-Arrastia et al., 2014) and 6-months intervals (Mondello, Akinyi, et al., 2012). Two recent studies report the same sensitivity of 100% and specificities of 21% and 39% (Mondello et al., 2017).

Clinical Utility and Validity

Welch et al (2016) evaluated three serum biomarkers' (glial fibrillary acidic protein [GFAP], ubiquitin C-terminal hydrolase-L1 [UCH-L1] and S100B measured within 6 h of injury) ability to differentiate CT-negative and CT-positive findings. They found that "UCH-L1 was 100% sensitive and 39% specific at a cutoff value >40 pg/mL. To retain 100% sensitivity, GFAP was 0% specific (cutoff value 0 pg/mL) and S100B had a specificity of only 2% (cutoff value 30 pg/mL). All three biomarkers had similar values for areas under the receiver operator characteristic curve: 0.79 for GFAP, 0.80 for UCH-L1, and 0.75 for S100B. Neither GFAP nor UCH-L1 curve values differed significantly from S100B. In our patient cohort, UCH-L1 outperformed GFAP and S100B when the goal was to reduce CT use without sacrificing sensitivity. UCH-L1 values <40 pg/mL could potentially have aided in eliminating 83 of the 215 negative CT scans" (Welch et al., 2016). However, the authors note that further research is needed.

Wang et al. (2018) reported on the usage of TBI serum and CSF biomarkers as prognostic tools in the ED, neurointensive care unit, and out-of-hospital settings. In the case of mTBI, the researchers stated the similar biomarkers could aid in predicting any development of persistent post-concussive syndrome, including S100B, GFAP, and UCH-L1. Within 12-36 hours from TBI in neurointensive care units, it was found that serum levels of 100B correlate with patient outcomes, and S100B serum levels > 0.7ng/mL correlate with 100% mortality. GFAP modestly correlates with poor outcomes, and "serum GFAP levels were also significantly higher in patients who died or had an unfavorable outcome and have predicted neurological outcome at 6 months." It was also shown in other studies that GFAP and UCHL-1 proteins outperformed S100B in predicting poor outcomes, and the two together "predicate the recovery and unfavorable outcome by distinguishing patients with GOS [Glasgow Outcome Score] 1-3 from patients with GOS 4-5" (Mondello et al., 2016; Takala et al., 2016; Wang et al., 2018).

Gan et al. (2019) evaluated TBI serum biomarkers for four clinical situations: "detecting concussion, predicting intracranial damage after mild TBI (mTBI), predicting delayed recovery after mTBI, and predicting adverse outcome after severe TBI (sTBI)". A total of 200 publications (61722 "observations") were included. For concussion detection, 9 unique publications addressing 15 biomarkers and 946 observations were identified. Four panels ("copeptin, galectin-3, and MMP-9; GFAP and UCH-L1; 10 metabolites; and 17 metabolites") were found to have areas under the curve (AUC) of over 0.9. For evaluation of necessity of CT scan after TBI, 56 publications, 24 biomarkers, and 23316 observations were identified. S-100B (30 publications, 8464 observations) was found to have an AUC of 0.723 and GFAP/GFAP-BDP (16 publications, 2040 observations) was found to have an AUC of 0.831. For evaluation of delayed recovery after mTBI, 44 publications, 29 biomarkers, and 13291 observations were identified. S-100B (24 publications, 2800 observations) had an AUC of 0.691; GFAP's AUC was 0.716 (17 publications, 1959 observations). Finally, for evaluation of poor outcome after sTBI, S-100B (25 publications, 3712 observations) was rated at AUC of 0.762, and GFAP (10 publications, 2448

observations) was rated at AUC of 0.749. Neuron-specific enolase (9 publications, 911 observations) was rated at AUC of 0.715 (Gan et al., 2019).

Korley et al. (2022) investigated the prognostic value of glial fibrillary acidic protein (GFAP) and ubiquitin C-terminal hydrolase L1 (UCH-L1) in traumatic brain injuries in a study called TRACK-BTI. The prognostic accuracy of the two biomarkers was studied amongst 2552 participants. Participants were 17 years and older and had been evaluated for TBI. All patients were given a head CT during evaluation. Participants had plasma samples taken on the day of injury (for measurement of GFAP and UCH-L1). In the results, of the 1696 participants with brain injury (data available at baseline and at 6 months), 120 (7.1%) died, 235 (13.9%) had unfavorable outcomes, and 561 (33.1%) recovered fully. The area under the curve of GFAP for predicting death at 6 months in all patients was .87 (95% CI 0.83-0.91), for unfavorable out come was 0.86 (0.83-0.89), and for incomplete recovery was 0.62 (0.59-0.64). The AUC for UHC-L1 was 0.89 (95% CI 0.86-0.92) for prediction of death, 0.86 (0.84-0.89) for unfavorable outcome, and 0.61 (0.59-0.64) for incomplete recovery at 6 months. Additionally, "Among participants with GCS [Glasgow Coma Scale] score of 3–12 (n=353), adding GFAP and UCH-L1 (alone or combined) to each of the three International Mission for Prognosis and Analysis of Clinical Trials in traumatic brain injury models significantly increased their AUCs for predicting death (AUC range 0·90–0·94) and unfavourable outcome (AUC range 0·83–0·89). The authors concluded, "GFAP and UCH-L1 plasma concentrations have good to excellent prognostic value for predicting death and unfavourable outcome, but not for predicting incomplete recovery at 6 months" (Korley et al., 2022).

In January 2021, Abbott Laboratories received FDA 510(K) clearance for the i-STAT™ Alinity™ handheld device, which would help evaluate mTBIs. It simultaneously measures UCH-L1 and GFAP in blood and produces results in 15 minutes once a plasma sample is inserted. It has a sensitivity of 95.8% and a >99% negative predictive value. Abbott Laboratories states that this blood test's availability "could help eliminate wait time in the emergency room and could reduce the number of unnecessary CT scans by up to 40%." The company is also working on a whole blood test, and has received breakthrough designation to create a TBI test that runs "on its Alinity™ and ARCHITECT® core laboratory instruments" (Abbott Laboratories, 2021).

In March 2023, Abbott Laboratories received FDA clearance for the Alinity® i TBI test that measures two biomarkers in the blood— C-terminal hydrolase L1 (UCH-L1) and glial fibrillary acidic protein. Like the i-STAT™ Alinity™, this test is intended for use in adults who are suspected of having mild traumatic brain injury, such as adults who present to the hospital within 12-hours of a concussion or suspected mTBI. Initial studies show the test provides results with 96.7% sensitivity and 99.4% negative predictive value. After a blood draw, results are available within 18 minutes and the test is run on Abbott's Alinity™ i platform (MPR, 2023).

Guidelines and Recommendations

American College of Emergency Physicians recommended consideration could be given to not performing a CT (Level C) in mild TBI patients without significant extracranial injuries and a serum S100β of level less than 0.1µg/L measured within 4 h of injury (Jagoda et al., 2009).

Centers for Disease Control (CDC, 2016) reaffirmed the 2008 ACEP recommendation in 2016. However, in 2018, the CDC remarked that "Health care professionals should not use biomarkers outside of a research setting for the diagnosis of children with mTBI", noting that there is insufficient evidence to recommend any of the studied biomarkers for mTBI diagnosis in children. The CDC identified S100B, tau protein, autoantibodies against glutamate receptors and oxide metabolites, neuronal ubiquitin C-

terminal hydrolase-L1, and glial fibrillary acidic protein biomarker levels as biomarkers that have been studied for concussion evaluation (Lumba-Brown et al., 2018).

The Veterans Administration and Department of Defense Practice Guideline for the Management of Concussion – mild Traumatic Brain Injury states that:

"Excluding patients with indicators for immediate referral, for patients identified by post-deployment screening or who present to care with symptoms or complaints potentially related to brain injury, we suggest *against* using the following tests to establish the diagnosis of mTBI or direct the care of patients with a history of mTBI:

a. Neuroimaging
b. Serum biomarkers, including S100 calcium-binding protein B (S100-B), glial fibrillary acidic protein (GFAP), ubiquitin carboxyl-terminal esterase L1 (UCH-L1), neuron specific enolase (NSE), and α-amino-3-hydroxy-5- methyl-4-isoxazolepropionic acid receptor (AMPAR) peptide
c. Electroencephalogram (EEG)" (VA/DoD, 2021).

The consensus statement from **American College of Sports Medicine (ACSM), American Academy of Family Physicians (AAFP), American Academy of Orthopedic Surgeons (AAOS), American Medical Society for Sports Medicine (AMSSM), American Orthopedic Society for Sports Medicine (AOSSM), and the American Osteopathic Academy of Sports Medicine (AOASM)** (Herring et al., 2011) states that: "Investigation in the area of biomarkers (e.g., S-100 proteins, neuron specific enolase, tau protein) is inconclusive for identifying individuals with concussion and represents research that may one day be clinically applicable."

The **American Academy of Pediatrics (AAP)** acknowledges that biomarkers such as "S100β, glial fibrillary acidic protein, neuron-specific enolase, τ, neurofilament light protein, amyloid β, brain-derived neurotrophic factor, creatine kinase and heart-type fatty acid binding protein, prolactin, cortisol, and albumin" have all been investigated in concussion evaluation, but none of these biomarkers have been used in clinical settings (AAP, 2018; Halstead et al., 2018).

The **National Institute for Health and Care Excellence (NICE)** guidelines regarding "assessment and early management of head injury in children, young people and adults" do not mention any serum biomarkers for evaluation of head injuries (NICE, 2019).

The **American Medical Society for Sports Medicine** notes that fluid biomarkers (blood, saliva, and cerebrospinal fluid) in diagnosis of sports-related concussion is under active investigation, but states that overall evidence level is "low". The Society writes that more studies are needed to determine their clinical utility. The Society also acknowledges the FDA approval of the "two-protein brain trauma indicator with glial fibrillary acidic protein and ubiquitin carboxy-terminal hydrolase L1 (UCHL1), and clinical use of S100 calcium-binding protein b (s100b) in Europe," but remark that neither of these tests have a role in diagnosis or management of a sports-related concussion (Harmon et al., 2019).

The **American Congress of Rehabilitation Medicine Brain Injury Interdisciplinary Special Interest Group Mild TBI Task Force** published a synthesis of practice guidelines for "Management of Concussion and Mild Traumatic Brain Injury." In it, they note that the Scandinavian Neurotrauma Committee guidelines recommend that "S100B values of <0.10 mg/L, if sampled within 6 hours of injury, can help rule out the need for CT in patients younger than 65 years with a Glasgow Coma Scale score of 14 or a Glasgow Coma Scale score of 15 with loss of consciousness or repeated vomiting". However, they also remark that neither GFAP nor C-terminal hydrolase-L1 have been incorporated into any

published clinical practice guidelines. Further, the task force notes that the biomarkers' incremental value over established clinical decision rules (such as the Canadian CT head rule) is unknown.

The task force also states that "At present, there is no objective biomarker to determine mTBI resolution" (Silverberg et al., 2020).

The **International Traumatic Brain Injury Research (InTBIR) Initiative** states that there "remains a critical need for more accurate diagnostic and prognostic tools in TBI. The development and validation of genomic, proteomic, and imaging biomarkers will be essential for tackling TBI heterogeneity and moving towards precision medicine. The heterogeneous nature of traumatic brain injury presents a major challenge to biomarker identification, validation, and clinical application."

In a statement on genomic screening, they note that a genome-scale wide approach hasn't gained traction over identifying single candidate biomarkers, and that "regardless of the method by which a candidate biomarker is identified, appropriate testing and validation is crucial to accurately assess a biomarker's predictive/diagnostic potential."

Regarding specific biomarkers, they state, "Proteins highly specific to astroglial overexpression and injury, S100B and glial fibrillary acidic protein (GFAP) are logical choices for investigation. S100B is a calcium-binding protein found in astrocytes, the levels of which are elevated in response to neural injury or inflammation. A number of clinical studies have shown that elevated serum levels of S100B correlate with poor outcome after TBI, but S100B has also been shown to be elevated in response to other inflammatory/traumatic processes in the absence of TBI." Furthermore, "In the case of S100B, although it has been shown to be highly sensitive to brain trauma, it lacks specificity for TBI because it is also released from extracerebral tissue and can be elevated in response to numerous other non-CNS injuries."

Regarding GFAP, they note it has been "suggested that it may serve as a marker of focal lesions and intracranial bleeding, but may not be adequately sensitive to axonal injury. Unlike GFAP, the protease ubiquitin C-terminal hydrolase-L1 (UCH-L1) has been shown to be suggestive of diffuse injuries, and appears to be a promising TBI biomarker candidate in its own right. Taken together, these observations suggest that simultaneous assessment of biomarkers reflecting different pathophysiological mechanisms and injury types would provide complementary information and might increase diagnostic and prognostic accuracy, hence enabling clinicians to stratify risk more effectively among TBI patients"(Huie et al., 2021).

Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: http://www.cms.gov/medicare-coverage-database/overview-and-quick-search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

On Jan 8, 2021, with 510(K) clearance, the FDA approved marketing ofi-STAT TBI Plasma Cartridge with the i-STAT™ Alinity™ System from Abbott Laboratories. This brain trauma assessment test is intended

for *in vitro* diagnostic use to aid in evaluating patients, 18 years of age or older, with suspected mTBI (Glasgow Coma Scale score 13-15) within 12 hours of injury with other clinical information to assess the need for radiologic imaging (CT, MRI). A result from this test is associated with the absence or presence of acute traumatic intracranial lesions seen on a head CT scan, but is not intended for use in point of care settings (FDA, 2021). In March 2023, the FDA approved Abbott's Alinity® i TBI lab test as a complement to the i-STAT™ Alinity™ System. According to Abbott, the test measures ubiquitin C-terminal hydrolase L1 (UCH-L1) and glial fibrillary acidic protein; the test assesses whether there are elevated concentrations of these biomarkers in the blood. While the i-STAT™ Alinity™ System is the first rapid hand-held test that measures biomarkers in plasma, the Alinity® i TBI test is a blood test run on Abbott's Alinity® i instrument (MPR, 2023).

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
83516	Immunoassay for analyte other than infectious agent antibody or infectious agent antigen; qualitative or semiquantitative, multiple step method
84999	Unlisted chemistry procedure

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

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Serum Testing for Hepatic Fibrosis in the Evaluation and Monitoring of Chronic Liver Disease

Policy Number: AHS – G2110 – Serum Testing for Hepatic Fibrosis in the Evaluation and Monitoring of Chronic Liver Disease	Prior Policy Name and Number, as applicable: • AHS – G2110 – Multianalyte Assays with Algorithmic Analysis for the Evaluation and Monitoring of Patients with Chronic Liver Disease • AHS – G2110 – Serum Marker Panels for Hepatic Fibrosis in the Evaluation and Monitoring of Chronic Liver Disease
Initial Presentation Date: 09/18/2015 Effective Date: February 1, 2025	

Policy Description

Chronic liver disease (CLD) refers to a wide range of liver pathologies that include inflammation (chronic hepatitis), liver cirrhosis, and hepatocellular carcinoma.

Hepatic fibrosis is associated with a cycle of extracellular matrix deposition and degradation. Biomarkers of extracellular matrix turnover are used to directly assess fibrosis and, theoretically, to monitor progression or regression (Valva et al., 2016). These markers include several glycoproteins, members of the collagen family, collagenases and their inhibitors, and several cytokines involved in the fibrogenic process (Valva et al., 2016). The markers may be utilized individually, as well as in panel combinations (Parikh et al., 2017).

Related Policies

Policy Number	Policy Title
AHS-G2036	Hepatitis Testing
AHS-G2124	Serum Tumor Markers for Malignancies
AHS-G2173	Gamma-glutamyl Transferase Testing in Adults

Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) For individuals with hepatitis C, hepatitis B, metabolic dysfunction-associated steatotic liver disease (MASLD) (including metabolic dysfunction-associated steatohepatitis [MASH]), **or** alcoholic hepatitis, the use of the following multianalyte assays with algorithmic analysis to distinguish hepatic cirrhosis from non-cirrhosis **MEETS COVERAGE CRITERIA** once every 6 months:
   a) ELF™(ELFTM).
   b) FibroTest®.
   c) HBV FibroSURE®.
   d) HCV FibroSURE®.

2) For individuals with hepatitis C, hepatitis B, MASLD, **or** alcoholic hepatitis, the use of other multianalyte assays with algorithmic analysis (e.g., ASH FibroSURE®, LIVERFAStTM, NASH FibroSURE®, OWLiver®) **DOES NOT MEET COVERAGE CRITERIA**.

3) For individuals with liver disease not meeting the above criteria, the use of multianalyte assays with algorithmic analysis **DOES NOT MEET COVERAGE CRITERIA**.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

4) Except as previously described, the use of the following serum biomarkers in immunoassays and/or immunohistochemistry assays **DOES NOT MEET COVERAGE CRITERIA**:
   a) Signal-induced proliferation-associated 1 like 1 (SIPA1L1)
   b) microRNA (miRNA or miR) analysis, including but not limited to, the following:
      i) microRNA-21 (miRNA-21 or miR-21)
      ii) miRNA-29a (miR-29a)
      iii) miRNA-122 (miR-122)
      iv) miRNA-221 (miR-221)
      v) miRNA-222 (miR-222)
   c) Chitinase 3-like 1 (CHI3L1)

d) Hyaluronic acid

e) Type III procollagen (PCIII)

f) Type IV collagen

g) Laminin

h) Plasma caspase-generated cytokeratin-18

i) Micro-fibrillar associated glycoprotein 4 (MFAP4)

Table of Terminology

Term	Definition
AAFP	American Academy of Family Physicians
AASLD	American Association for the Study of Liver Diseases
AFP	Alpha-fetoprotein
AGA	American Gastroenterological Association
ALT	Alanine aminotransferase
ALT	Alanine transaminase
AST	Aspartate aminotransferase-to-platelet ratio index
AUC	Area under the curve
BMI	Body mass index
CDC	Centers for Disease Control and Prevention
CHBV	Chronic hepatitis B virus
CHC	Chronic hepatitis C
CHCV	Chronic hepatitis C virus infection
CK-18	Cytokeratin-18 fragments
CLD	Chronic liver disease
CMS	Centers for Medicare and Medicaid Services
EASD	European Association for the Study of Diabetes
EASL	European Association for the Study of Obesity
GGT	Gamma-glutamyl transferase
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
IDSA	Infectious Diseases Society of America
LDTs	Laboratory developed tests
MASH	Metabolic dysfunction-associated steatohepatitis
MASLD	Metabolic dysfunction-associated steatotic liver disease
MFAP4	Microfibrillar-associated protein 4
miRNA	Micro ribonucleic acid
MTX	Methotrexate
NAFLD	Nonalcoholic fatty liver disease
NASH	Nonalcoholic steatohepatitis
NICE	National Institute for Health and Care Excellence

NILTS	Non-invasive fibrosis tests
NIT	Non-invasive test
PT/INR	Prothrombin time/elevated international normalized ratio
SC	Standard care
SIPA1L1	Signal-induced proliferation-associated 1 like 1
SWE	Shear-wave elastography
TACE	Trans-arterial chemoembolization
TE	Transient elastography
US	Ultrasonography
USPSTF	United States Preventive Services Task Force
VCTE	Vibration controlled transient elastography
WHO	World Health Organization

Scientific Background

Fibrosis is a wound healing response in which damaged regions are encapsulated by an extracellular matrix. This is common in individuals with chronic liver injury but may be seen in other organs such as the kidneys or lungs. Chronic liver injury may be caused by numerous conditions, such as hepatitis or metabolic dysfunction-associated steatotic liver disease (MASLD) (formerly known as nonalcoholic fatty liver disease [NAFLD]), including metabolic dysfunction-associated steatohepatitis (MASH) (formerly known as nonalcoholic steatohepatitis [NASH]) (EASL, 2023), and progressive fibrosis may lead to cirrhosis (Friedman, 2024). Liver biopsy remains the gold standard for evaluation of chronic liver disease to monitor treatment and disease progression. However, this invasive procedure has several drawbacks including pain, bleeding, inaccurate staging due to sampling error, and variability of biopsy interpretation (Chin et al., 2016).

Serum biomarkers, such as the aspartate aminotransferase (AST) to platelet ratio (APRI), have been proposed as measures of hepatic fibrosis assessment, and numerous panels exist (Curry & Afdhal, 2024). These markers (and corresponding panels) may be categorized as "direct" or "indirect." Direct markers of fibrosis evaluate extracellular matrix turnover, and indirect markers signify changes in hepatic function. Direct biomarkers may be further subdivided by markers associated with matrix deposition, matrix degradation, or cytokines (and chemokines) associated with fibrogenesis. Procollagen I peptide, procollagen III peptide, type I collagen, type IV collagen, YKL-40 (chondrex), laminin, and hyaluronic acid, MMP-2, TIMP-1, -2, TGF-beta, TGF-alpha, and PDGF have all been proposed as direct measures of fibrosis. Indirect markers include serum aminotransferase levels, platelet count, coagulation parameters, gamma-glutamyl transferase (GGT), total bilirubin, alpha-2-macroglobulin, and alpha-2-globulin (haptoglobin) (Curry & Afdhal, 2024). Other markers have been investigated to be used independently or as part of these panels. The human microfibrillar-associated protein 4 (MFAP4) is located in extracellular matrix fibers and plays a role in disease-related tissue remodeling. Bracht et al. (2016) evaluated the "potential" of MFAP4 as a biomarker for hepatic fibrosis. A total of 542 patients were included, and the authors focused on differentiation of no to moderate (F0–F2) and severe fibrosis stages and cirrhosis (F3 and F4). In the "leave-one-out cross validation," a sensitivity of 85.8% and specificity of 54.9% was observed and the multivariate model yielded 81.3 % sensitivity and 61.5 % specificity. The authors suggested that "the combination of MFAP4 with existing tests might lead to a more accurate non-invasive diagnosis of hepatic fibrosis and allow a cost-effective disease management in the era of new direct acting antivirals" (Bracht et al., 2016).

Plasma caspase-generated cytokeratin-18 fragments (CK-18) have been proposed as a biomarker in the diagnosis and staging of nonalcoholic steatohepatitis (NASH). Cusi et al. (2014) studied the clinical value of CK-18. The authors studied the adipose tissue, liver, and muscle insulin resistance of 424 patients as well as liver fat (n = 275) and histology (n = 318). The authors found that median CK-18 levels were elevated in patients with verses without nonalcoholic fatty liver disease (NAFLD) (209 U/L vs. 122 U/L) or with verses without NASH (232 U/L vs. 170 U/L). The CK-18 area under curve to predict NAFLD, NASH, or fibrosis were 0.77, 0.65, and 0.68, respectively. The overall sensitivity/specificity for NAFLD, NASH and fibrosis were 63%/83%, 58%/68% and 54%/85%, respectively. CK-18 correlated most strongly with ALT (r=0.57) and adipose tissue IR (insulin-suppression of FFA: r=-0.43), but not with ballooning, body mass index, metabolic syndrome, or type 2 diabetes. The authors concluded, "Plasma CK-18 has a high specificity for NAFLD and fibrosis, but its limited sensitivity makes it inadequate as a screening test for staging NASH. Whether combined as a diagnostic panel with other biomarkers or clinical/laboratory tests may prove useful requires further study" (Cusi et al., 2014).

Likewise, Chitinase 3-like 1 (CHI3L1) has been proposed to be a better serum biomarker than hyaluronic acid, type III procollagen, type IV collagen, and laminin. CHI3L1 is preferentially expressed in hepatocytes over any other body tissue. Huang et al. (2015) investigated CHI3L1 in 98 patients with hepatitis B. The authors reported that CHI3L1 can be used to differentiate between early stages of liver fibrosis (S0-S2) from late stages (S3-S4) "with areas under the ROC curves (AUCs) of 0.94 for substantial (S2, S3, S4) fibrosis and 0.96 for advanced (S3, S4) fibrosis" (Huang et al., 2015). Wang et al. (2018) also report that CHI3L1 is a useful marker for the assessment of liver fibrosis before treatment and can also be used to monitor change during therapy.

MicroRNA (miRNA) sequences have also been proposed as a marker of liver function. MiRNA sequences often have roles in gene regulation and other cellular processes, so changes in these sequences may indicate a liver condition (Tendler, 2022). For example, Abdel-Al et al. (2018) investigated miRNA's association with Hepatitis C virus (HCV) patients. Forty-two patients with HCV and early-stage fibrosis, 45 patients with HCV and late-stage fibrosis, and 40 healthy controls were examined and the expression patterns of five miRNA sequences (miR-16, miR-146a, miR-214-5p, miR-221, and miR-222) were measured. The authors found miRNA-222 to have the highest sensitivity and specificity for both fibrosis groups, and all mi-RNA sequences except miRNA-214-5p were significantly upregulated in fibrosis. MiRNA-221 was also found to have significant positive correlations with miRNA-16 and miRNA-146a. The authors concluded that "the high sensitivity and specificity of miRNA-222 and miRNA-221 in late-stage fibrosis indicate promising prognostic biomarkers for HCV-induced liver fibrosis (Abdel-Al et al., 2018).

Multiple biomarkers may be combined into a panel. Panels may include a combination of direct markers, indirect markers, or markers from both categories. The most studied panels are the aspartate aminotransferase (AST) to platelet ratio (APRI), FibroTest/FibroSure, and Hepascore, although many more exist. FibroTest/FibroSure incorporates alpha-2-macroglobulin, alpha-2-globulin (haptoglobin), gamma globulin, apolipoprotein A1, GGT, and total bilirubin, age, and sex. HepaScore measures bilirubin, GGT, hyaluronic acid, alpha-2-macroglobulin, age, and sex. These panels have demonstrated some promising results, but Curry and Afdhal (2024) note that indeterminate outcomes are common. Furthermore, they state that no singular panel has emerged as the standard of care (Curry & Afdhal, 2024). Another test, known as the LIVERFASt™ by Fibronostics, utilizes a blood sample to measure 10 biomarkers; algorithm technology is used "to determine the fibrosis, activity and steatosis stages of the liver" (Fibronsotics, 2020). OWLiver® by CIMA Sciences, LLC, evaluates 28 metabolites from a blood sample. Relative concentrations of those biomarkers are analyzed together with two algorithms to generate a final OWLiver® score, which "indicates the probability of approximation of the patient's liver

status to a healthy liver / steatosis stage, a non-alcoholic steatohepatitis (NASH *) stage, or NASH and significant-advanced fibrosis (≥F2) stage" (CIMA Sciences, 2023).

Many combinations of biomarkers, and even combinations of panels, exist. For example, FibroMax combines FibroTest, SteatoTest, NashTest, ActiTest, and AshTest on the same result sheet and provides a more comprehensive estimation of the liver injury. This test measures 10 biomarkers which are as follows: GGT, total bilirubin, alpha-2-macroglobulin, apolipoprotein A1, haptoglobin, alanine aminotransferase (ALT), AST Transaminase, triglycerides, cholesterol, and fasting glucose (BioPredictive, 2019). Fouad et al. (2013) analyzed samples from 44 patients and found that FibroMax results were positively correlated with viral load by quantitative polymerase chain reaction and histopathological findings. Further, body mass index was significantly higher in steatotic patients and was significantly associated with the results on FibroMax (Fouad et al., 2013).

Clinical Utility and Validity

Berends et al. (2007) performed a study assessing FibroTest's (known as FibroSure in the United States) ability to detect methotrexate (MTX)-induced hepatic fibrosis. Twenty-four psoriasis patients that underwent a liver biopsy were included, and FibroTest identified 83 percent of the patients who had significant fibrosis. The authors suggested FibroTest may be used as part of monitoring MTX-induced fibrosis (Berends et al., 2007).

Kwok et al. (2014) performed a meta-analysis of non-invasive assessments of NASH. The authors identified nine studies for transient elastography (TE) and 11 for cytokeratin-18 (CK-18). The pooled sensitivities and specificities for TE to diagnose F ≥ 2, F ≥ 3, and F4 disease were 79% and 75%, 85% and 85%, and 92% and 92%, respectively. CK-18 was found to have a pooled sensitivity of 66% and specificity of 82% in diagnosing NASH. The authors concluded that "At present, serum tests and physical measurements such as TE come close as highly accurate non-invasive tests to exclude advanced fibrosis and cirrhosis in NAFLD patients. CK18 has moderate accuracy in diagnosing NASH, while other biomarkers have not been extensively studied" (Kwok et al., 2014).

Gao et al. (2018) compared aspartate amino transferase–to-platelet ratio index (APRI), the Fibrosis-4 index (FIB-4), transient elastography (TE), and two-dimensional (2D) shear-wave elastography (SWE). A total of 402 patients with chronic hepatitis B were included. 2D-SWE was found to have the highest area under the curve (AUC), with 0.87 compared to APRI's 0.70, TE's 0.80, and FIB-4's 0.73 (Gao et al., 2018).

Dong et al. (2018) compared the performance of several biomarkers (serum hyaluronan (HA), procollagen type III N-terminal peptide (PIIINP), type IV collagen (IVC), laminin (LN), ALT, AST) to transient elastography (FibroScan). Seventy patients with hepatitis B underwent a liver biopsy. Fibrosis was found in 24 patients. The correlation of serum levels with fibrosis stage are as follows: 0.468 (HA), 0.392 (PIIINP), 0.538 (IVC), 0.213 (LN), 0.350 (ALT), 0.375 (AST). The authors found that the combination of all five biomarkers yielded a superior diagnostic performance (area under curve: 0.861) compared to all five alone (Dong et al., 2018).

A pilot study of the FM-fibro index was performed with 400 patients enrolled, and the FM-fibro index, CA-fibro index, and European Liver Fibrosis panel (ELF) were compared with respect to estimating prognosis of patients with NAFLD. Three separate biomarkers comprise the FM-fibro index: type IV collagen 7S, hyaluronic acid, and vascular cell adhesion molecule-1. The area under the curve was 0.7093 for the CA-fibro index, 0.7245 for ELF, and 0.7178 (type IV collagen 7S)/0.7095 (hyaluronic acid)/0.7065 (vascular cell adhesion molecule-1) (Itoh et al., 2018). The sensitivity and specificity of the FM-fibro index

for predicting NASH-related fibrosis was 0.5359/0.5210/0.4641 and 0.8333/0.8182/0.8788, respectively (Itoh et al., 2018). The accuracy of the FM-fibro index was not significantly different from that of the CA-fibro index and the ELF panel.

Patel et al. (2018) performed a retrospective study focusing on fibrosis scoring systems to identify NAFLD. A total of 329 patients (296 NAFLD, 33 controls) were included. The following indices were studied: "NAFLD fibrosis score (NFS), fibrosis-4 calculator (FIB-4), aspartate aminotransferase-to-alanine aminotransferase ratio (AST/ALT ratio), AST-to-platelet ratio index (APRI), and body mass index, AST/ALT ratio, and diabetes (BARD) score by age groups" (Patel et al., 2018). NFS and FIB-4 were found to best predict advanced fibrosis with areas under curve of 0.71-0.76 and 0.62-0.80 respectively. However, the authors concluded that "While NFS and FIB-4 scores exhibit good diagnostic accuracy, FIB-4 is optimal in identifying NAFLD advanced fibrosis in the VHA. Easily implemented as a point-of-care clinical test, FIB-4 can be useful in directing patients that are most likely to have advanced fibrosis to GI/hepatology consultation and follow-up" (Patel et al., 2018).

Kim et al. (2017) evaluated the "association between plasma miR-122 [microRNA-122] and treatment outcomes following transarterial chemoembolization (TACE) in hepatocellular carcinoma patients." A total of 177 patients were included, and miR-122 levels were measured; the researchers found that 112 patients exhibited TACE refractoriness. Multivariate analyses showed that tumor number (hazard ratio [HR], 2.51) and tumor size (HR, 2.65) can independently predict overall TACE refractoriness. High miR-122 expression (> 100) was associated with early TACE refractoriness (within 1 year; HR, 2.77; 95% CI,) together with tumor number (HR, 22.73) and tumor size (HR, 4.90). Univariate analyses showed that high miR-122 expression tends to be associated with poor liver transplantation-free survival (HR, 1.42). However, this was statistically insignificant in multivariate analysis. The authors concluded that "High expression levels of plasma miR-122 are associated with early TACE refractoriness in HCC patients treated with TACE" (Kim et al., 2017).

Suehiro et al. (2018) performed a study analyzing "the importance of serum exosomal miRNA expression levels in hepatocellular carcinoma (HCC) patients that underwent transarterial chemoembolization (TACE)." Seventy-five patients underwent TACE. Exosomal miR-122 expression levels significantly decreased after TACE. The expression levels of exosomal miR-122 before TACE were shown to correlate significantly with AST (r=0.31) and ALT (r=0.33) levels. According to the median relative expression of miR-122 after TACE/before TACE (miR-122 ratio) in liver cirrhosis patients (n=57), the patients with a higher miR-122 ratio had significantly longer disease-specific survival compared with that of the patients with the lower miR-122 ratio. A lower exosomal miR-122 ratio (HR 2.720) was associated with the disease-specific survival. The authors concluded that "the exosomal miR-122 level alterations may represent a predictive biomarker in HCC patients with liver cirrhosis treated with TACE" (Suehiro et al., 2018).

Kar et al. (2019) analyzed the performance of biomarkers implicated in hepatic inflammation. The authors enrolled 52 patients with NAFLD/NASH and evaluated the following biomarkers: IL-6, CRP, TNFα, MCP-1, MIP-1β, eotaxin, and VCAM-1. Serum IL-6 was found to be increased in patients with advanced fibrosis (2.71 pg/mL in fibrosis stages 3 and 4 compared to 1.26 pg/mL in stages 1-2 and 1.39 pg/mL in stage 0), but there were no other significant differences in CRP, TNFα, MCP-1, MIP-1β. VCAM-1 was noted to have increased by 55% over the mild fibrosis group and 40% over the no fibrosis group. VCAM-1 was also observed to have an area under curve of 0.87. The authors suggested that the "addition of biomarkers such as IL-6 and VCAM-1 to panels may yield increased sensitivity and specificity for staging of NASH" (Kar et al., 2019).

Srivastava et al. (2019) performed a cost-benefit analysis of non-invasive fibrosis tests (NILTS) for nonalcoholic fatty liver disease (NAFLD). The authors compared the current standard of care, FIB-4, and the Enhanced Liver Fibrosis (ELF) panel. The simulations consisted of 10000 NAFLD patients. Standard care (SC) was compared to the following four scenarios: "FIB-4 for all patients followed by ELF test for patients with indeterminate FIB-4 results; FIB-4 followed by fibroscan for indeterminate FIB-4; ELF alone; and fibroscan alone." The authors identified the following observations: "Introduction of NILT increased detection of advanced fibrosis over one year by 114, 118, 129 and 137% compared to SC in scenarios 2, 3, 4 and 5 respectively with reduction in unnecessary referrals by 85, 78, 71 and 42% respectively. Total budget spend [sic] was reduced by 25.2, 22.7, 15.1 and 4.0% in Scenarios 2, 3, 4 and 5 compared to £670 K at baseline." The authors suggested that the "use of NILT in primary care can increase early detection of advanced liver fibrosis and reduce unnecessary referral of patients with mild disease and is cost efficient" (Srivastava et al., 2019).

Weis et al. (2019) evaluated miRNA expression's ability to distinguish between HCC and cirrhosis. Sixty patients with chronic hepatitis C (CHC) were divided into three groups; 20 with fibrosis stages 0-2, 20 with cirrhosis, and 20 with cirrhosis and HCC. A total of 372 miRNA sequences were measured. The authors found that a theoretical panel consisting of miRNA-122-5p, miRNA-486-5p, and miRNA-142-3p distinguished HCC from cirrhosis (area under the curve [AUC]= 0.94; sensitivity = 80%, specificity = 95%) outperforming alpha-fetoprotein (AFP) (AUC = 0.64). Another theoretical panel of miRNA-122-5p and miRNA-409-3p distinguished cirrhosis from mild disease (AUC = 0.80; sensitivity = 85%, specificity = 70%). The authors concluded that "MicroRNAs have great potential as diagnostic biomarkers in CHC, particularly in HCC where they outperform the only currently-used biomarker, AFP" (Weis et al., 2019).

Both Parikh et al. (2017) and Kaswala et al. (2016) performed studies evaluating the diagnostic accuracy of non-invasive markers for liver conditions. Parikh et al. (2017) focused on chronic hepatitis B virus (HBV) infections while Kaswala et al. (2016) studied nonalcoholic fatty liver. Tables detailing their summarized findings are listed below:

Diagnostic accuracy of most commonly used non-invasive fibrosis (≥F2) tests in chronic HBV infection from (Parikh et al., 2017)

Test	*Cut-off*	*AUROC*	*Sensitivity (%)*	*Specificity (%)*
Indirect markers				
FIB-4 index (high cut-off)	3.25	N/A	16.2	73.6
FIB-4 index (low cut-off)	1.45–1.62	0.78	65	77
APRI (low cut-off)	0.5	0.79	84	41
APRI (high cut-off)	1.5		49	84
Forns index (low cut-off)	3.11	0.68	91.4	31.5
Forns index (high cut-off)	5.11	N/A	42.5	75
Direct markers				
Hyaluronic acid	113–203	0.73	63–80	78–94
Hepascore	0.32	0.75	74	69
Fibrotest	0.38	0.77	65	78
Fibrometer	0.47	0.84	73	80
ELF	8.75	0.8	NA	NA

Diagnostic accuracy of most commonly used non-invasive fibrosis tests in nonalcoholic fatty liver (NAFL) from (Kaswala et al., 2016)

Test	*Cut-off*	*AUROC*	*Sensitivity (%)*	*Specificity (%)*
AST/ALT ratio	1	0.83	21	90
AST to platelet ratio index (low cutoff)	0.45	0.67–0.94	30	93
AST to platelet ratio index (high cutoff)	1.5			
BAAT score	2	0.84	71	80
BARD	2	0.8	86.8	32.5
ELF test	8.5–11.35	0.82–0.90	80	90
FibroMeter (low cutoff)	F3: 0.61	0.90–0.94	81	84
FibroMeter (high cutoff)	0.71			
FibroTest (low cutoff)	0.3	0.81–0.92	15–77	77–90
FibroTest (high cutoff)	0.7			
FIB-4 (low cutoff)	1.3–1.92	0.88	26–74	71–98
FIB-4 (high cutoff)	3.25			
Hepascore	0.37	0.81	75.5	84.1
	0.7	0.9	87	89
NAFLD (low cutoff)	−1.45	0.81	51	96
NAFLD (high cutoff)	0.67			

AST- aspartate aminotransferase; APRI- AST to platelet ratio; BAAT- body mass index (BMI), age, alanine aminotransferase (ALT), triglycerides; BARD- BMI, AST/ALT ratio, diabetes; ELF- Enhanced Liver Fibrosis panel; FIB-4- Fibrosis-4 index; NAFLD – Nonalcoholic fatty liver disease

Bril et al. (2019) assessed the performance of the FibroTest, along with other tests which measure steatosis, necrosis, and inflammation (the SteatoTest, ActiTest, NashTest), in a cohort of patients with type 2 diabetes. A total of 220 diabetic patients participated in this study. Plasma samples from each participant were used for the FibroTest. The researchers note that "Regarding the FibroTest score, its performance to identify patients with moderate or advanced fibrosis was 0.67" (Bril et al., 2019). The authors concluded that "Non-invasive panels for the diagnosis of steatosis, NASH and/or fibrosis, which were developed and validated in non-diabetic cohorts, underperformed when applied to a large cohort of patients with T2DM [type 2 diabetes mellitus]" (Bril et al., 2019)

In a metanalysis, seven studies reported the accuracy of FibroTest™ in nonalcoholic fatty liver disease (NAFLD) patients. The mean AUC was 0.77, mean sensitivity was 0.72, and mean specificity was 0.69. Due to poor AUC, sensitivity, and specificity values, FibroTest™ did not meet the minimally acceptable performance level in detecting significant, advanced, or any fibrosis. However, diagnostic accuracy of FibroTest™ was more promising in detecting cirrhosis, with an AUC of 0.92. The author states that in primary care settings which have a low disease prevalence, FibroTest™ can have a high negative predictive value, based on sensitivities between 0.90 and 0.98, demonstrating its ability to rule out advanced fibrosis in NAFLD patients. However, the test does have low specificity, leading to a considerable number of false positive results, which can lead to invasive and expensive follow-up tests. Overall, "this analysis showed that by optimizing sensitivity to values above 0.90, the test could result in

high NPVs (>90%) in settings with low prevalence of disease, such as primary and secondary care settings, but with relatively low PPVs (11–61%)" (Vali et al., 2021).

Chow et al. (2023) conduced a systematic review of society guidelines to compare recommendations for screening, diagnosis, and assessment of NAFLD. Two researchers independently extracted key information from 20 guidelines published between 2015 and 2022. "No guidelines recommended routine screening for NAFLD, while 14 guidelines recommended case finding in high-risk groups," but guidelines differed on cutoffs and interpretations of high-risk results. Overall, the authors concluded that "despite their differences, all guidelines recognize the utility of NITs and recommend their incorporation into the clinical assessment of NAFLD" (Chow et al., 2023).

Vali et al. (2023) studied the diagnostic accuracy of non-invasive biomarkers in detecting NASH and clinically significant fibrosis in patients with NAFLD. The researchers studied 17 biomarkers and multimarker scores. A total of 1430 participants with NAFLD were included from 13 countries in Europe. "For people with NASH and clinically significant fibrosis, no single biomarker or multimarker score significantly reached the predefined AUC 0·80 acceptability threshold." For the detection of advanced fibrosis, SomaSignal (AUC 0·90), ADAPT (AUC 0·85), and FibroScan liver stiffness measurement (AUC 0·83) all reached acceptable accuracy. "With 11 of 17 markers, histological screen failure rates could be reduced to 33% in trials if only people who were marker positive had a biopsy for evaluating eligibility." The authors concluded that "none of the single markers or multimarker scores achieved the predefined acceptable AUC for replacing biopsy in detecting people with both NASH and clinically significant fibrosis. However, several biomarkers could be applied in a prescreening strategy in clinical trial recruitment" (Vali et al., 2023)

Guidelines and Recommendations

American Academy of Family Physicians (AAFP)

The 2019 AAFP guideline lists viral hepatitis, alcoholic liver disease, and nonalcoholic steatohepatitis as the most common causes of cirrhosis. They state that "common serum and ultrasound-based screening tests to assess fibrosis include the aspartate transaminase to platelet ratio index score, Fibrosis 4 score, FibroTest/FibroSure, nonalcoholic fatty liver fibrosis score, standard ultrasonography, and transient elastography. Generally noninvasive tests are most useful in identifying patients with no to minimal fibrosis or advanced fibrosis. Chronic liver disease management includes directed counseling, laboratory testing, and ultrasound monitoring" (AAFP, 2019).

In regards to the monitoring of patients post-diagnosis and staging, "For patients with cirrhosis, a basic metabolic panel, liver function tests, complete blood count, and PT/INR should be completed every six months to recalculate Child-Pugh and Model for End-Stage Liver Disease scores" (AAFP, 2019).

American Association for the Study of Liver Diseases (AASLD)

The 2015 AASLD and Infectious Diseases Society of America (IDSA) recommendations for testing, managing, and treating adults infected with hepatitis C virus stated that "Recently, noninvasive tests to stage the degree of fibrosis in patients with chronic HCV infection include models incorporating indirect serum biomarkers (routine tests such as aspartate transaminase, alanine transaminase [ALT], and platelet count), direct serum biomarkers (components of the extracellular matrix produced by activated hepatic stellate cells), and vibration-controlled transient liver elastography. No single method is recognized to have high accuracy alone, and the results of each test must be interpreted carefully." The guidelines further stated that "although liver biopsy is the diagnostic standard, sampling error and observer

variability limit test performance, particularly when inadequate sampling occurs. In addition, the test is invasive and minor complications are common, limiting patient and practitioner acceptance. Serious complications such as bleeding, although rare, are well recognized." The guidelines further recommend that for patients who fail to achieve a sustained virological response, "disease progression assessment every 6 months to 12 months with a hepatic function panel, complete blood count, and international normalized ration" (AASLD-IDSA, 2015).

The 2018 AASLD and Infectious Diseases Society of America (IDSA) recommendations for HCV testing stated that "evaluation for advanced fibrosis using liver biopsy, imaging, and/or noninvasive markers is recommended for all persons with HCV infection, to facilitate an appropriate decision regarding HCV treatment strategy and to determine the need for initiating additional measures for the management of cirrhosis (eg, hepatocellular carcinoma screening). Rating: Class I, Level A" (AASLD-IDSA, 2019).

The 2018 AASLD update (Terrault et al., 2018) on prevention, diagnosis, and treatment of chronic hepatitis B states that:

For monitoring patients with a chronic HBV infection, who are not currently on treatment, "Alternative methods to assess fibrosis are elastography (preferred) and liver fibrosis biomarkers (e.g., FIB-4 or FibroTest). If these noninvasive tests indicate significant fibrosis (≥F2), treatment is recommended."

The 2018 AASLD practice guidelines (Chalasani et al., 2017) on the diagnosis and management of nonalcoholic fatty liver disease recommend:

- "In patients with NAFLD, metabolic syndrome predicts the presence of steatohepatitis, and its presence can be used to target patients for a liver biopsy."
- "NFS or FIB-4 index are clinically useful tools for identifying NAFLD patients with higher likelihood of having bridging fibrosis (stage 3) or cirrhosis (stage 4)."
- "Vibration controlled transient elastography or magnetic resonance elastography are clinically useful tools for identifying advanced fibrosis in patients with NAFLD. "

The AASLD does not mention miRNA for assessment in liver disease.

A 2019 update from the AASLD and IDSA states that "Noninvasive tests using serum biomarkers or imaging allow for accurate diagnosis of cirrhosis in most individuals" and frequently used noninvasive methods to estimate liver disease severity include "serum fibrosis marker panels" (AASLD-IDSA, 2019). Further, regarding recommendations for counseling persons with an active HCV infection, the guideline recommend that "Evaluation for advanced fibrosis using noninvasive markers or liver biopsy, if required, is recommended for all persons with HCV infection to facilitate an appropriate decision regarding HCV treatment strategy, and to determine the need for initiating additional measures for cirrhosis management (e.g., hepatocellular carcinoma screening)" (AASLD-IDSA, 2019).

In a 2021 update, AASLD discussed changes in liver biochemistry during normal pregnancy. AASLD states that an "elevation in aminotransferases, bilirubin, or bile acids in pregnancy is abnormal and requires investigation. Evaluation in pregnant patients must include a thorough history (including travel, environmental, and drug exposures), physical examination, and focused serologic testing. Hepatic ultrasonography (US) is the favored initial imaging modality. Diagnosis can usually be determined without liver biopsy" (Sarkar et al., 2021).

In 2023, the AASLD and IDSA stated "For initial HCV testing, the Guidance Panel recommends HCV antibody screening with reflex HCV RNA testing to establish the presence of active infection (as opposed to spontaneous or treatment-induced viral clearance)" (Bhattacharya et al., 2023).

American Gastroenterological Association (AGA)

The 2017 guidelines (Lim et al., 2017) on the Role of Elastography in the Evaluation of Liver Fibrosis state that:

- "In patients with chronic hepatitis C, the AGA recommends vibration controlled transient elastography, if available, rather than other nonproprietary, noninvasive serum tests (APRI, FIB-4) to detect cirrhosis."
- "In patients with chronic hepatitis B, the AGA suggests vibration controlled transient elastography (VCTE) rather than other nonproprietary noninvasive serum tests (ie, APRI and FIB-4) to detect cirrhosis."
- "The AGA makes no recommendation regarding the role of VCTE in the diagnosis of cirrhosis in adults with NAFLD."

In 2023, the AGA released an expert review of the role of noninvasive biomarkers in the evaluation and management of nonalcoholic fatty liver disease (Wattacheril et al., 2023). The AGA recommends:

- "NITs can be used for risk stratification in the diagnostic evaluation of patients with NAFLD.
- A Fibrosis 4 Index score <1.3 is associated with strong negative predictive value for advanced hepatic fibrosis and may be useful for exclusion of advanced hepatic fibrosis in patients with NAFLD.
- A combination of 2 or more NITs combining serum biomarkers and/or imaging-based biomarkers is preferred for staging and risk stratification of patients with NAFLD whose Fibrosis 4 Index score is >1.3.
- Use of NITs in accordance with manufacturer's specifications (eg, not in patients with ascites or pacemakers) can minimize risk of discordant results and adverse events.
- NITs should be interpreted with context and consideration of pertinent clinical data (eg, physical examination, biochemical, radiographic, and endoscopic) to optimize positive predictive value in the identification of patients with advanced fibrosis.
- Liver biopsy should be considered for patients with NIT results that are indeterminate or discordant; conflict with other clinical, laboratory, or radiologic findings; or when alternative etiologies for liver disease are suspected.
- Serial longitudinal monitoring using NITs for assessment of disease progression or regression may inform clinical management (ie, response to lifestyle modification or therapeutic intervention).
- Patients with NAFLD and NITs results suggestive of advanced fibrosis (F3) or cirrhosis (F4) should be considered for surveillance of liver complications (eg, hepatocellular carcinoma screening and variceal screening per Baveno criteria). Patients with NAFLD and NITs suggestive of advanced hepatic fibrosis (F3) or (F4), should be monitored with serial liver stiffness measurement; vibration controlled transient elastography; or magnetic resonance elastography, given its correlation with clinically significant portal hypertension and clinical decompensation."

World Health Organization (WHO)

In March 2015, the WHO released Guidelines for the Prevention, Care and Treatment of Persons with Chronic Hepatitis B Infection. In the section titled "Non-invasive Assessment of Liver Disease Stage at Baseline and during Follow up," the following is noted: aspartate aminotransferase (AST)-to-platelet ratio index (APRI) is recommended as the preferred non-invasive test (NIT) to assess for the presence of cirrhosis (APRI score >2 in adults) in resource-limited settings. Transient elastography (e.g., FibroScan) or FibroTest may be the preferred NITs in settings where they are available and cost is not a major constraint (WHO, 2015). In 2024, the WHO added a new recommendation for non-invasive test thresholds to establish the presence of significant fibrosis (≥F2) or cirrhosis (F4): "Evidence of significant fibrosis (≥F2) should be based on an APRI score of >0.5 or transient elastography value of >7.0 kPa, and cirrhosis (F4) should be based on clinical criteria (or an APRI score of >1.0 or transient elastography (FibroScan®) value of >12.5 kPa a)." The clinical features of decompensated cirrhosis are: "portal hypertension (ascites, variceal haemorrhage and hepatic encephalopathy), coagulopathy, or liver insufficiency (jaundice). Other clinical features of advanced liver disease/cirrhosis may include: hepatomegaly, splenomegaly, pruritus, fatigue, arthralgia, palmar erythema or oedema" (WHO, 2024).

In 2018, the WHO also published guidelines for management of patients with Hepatitis C. In it, they suggest "that aminotransferase/platelet ratio index (APRI) or FIB-4 be used for the assessment of hepatic fibrosis rather than other non-invasive tests that require more resources such as elastography or FibroTest." However, they do note that "FibroScan, which is more accurate than APRI and FIB-4, may be preferable in settings where the equipment is available and the cost of the test is not a barrier to testing."

The WHO does not mention miRNA as a tool for assessment of hepatitis (WHO, 2018).

United States Preventive Services Task Force (USPSTF)

The USPSTF published their final recommendation statement on Hepatitis C screening in adolescents and adults in 2020. THE USPSTF recommends "screening for hepatitis C virus (HCV) in adults aged 18 to 79" (grade B recommendation) (USPSTF, 2020).

National Institute for Health and Care Excellence (NICE)

NICE has released guidelines regarding chronic liver conditions. They note that the enhanced liver fibrosis test (ELF) may be considered in patients with NAFLD to test for advanced liver fibrosis. The ELF test should be offered to adults every three years and to children and young people every two years. (NICE, 2016).

European Association for the Study of the Liver (EASL), European Association for the Study of Diabetes (EASD) and European Association for the Study of Obesity

These joint guidelines include recommendations for fibrosis, mentioning ELF, FibroTest, NFS, and FIB-4. Their recommendations include the following:

- "Biomarkers and scores of fibrosis, as well as transient elastography, are acceptable non-invasive procedures for the identification of cases at low risk of advanced fibrosis/cirrhosis ($A2^{1,5}$). The combination of biomarkers/ scores and transient elastography might confer additional diagnostic accuracy and might save a number of diagnostic liver biopsies ($B2^{2,5}$)."
- "Monitoring of fibrosis progression in clinical practice may rely on a combination of biomarkers/scores and transient elastography, although this strategy requires validation ($C2^{3,5}$)."

- "The identification of advanced fibrosis or cirrhosis by serum biomarkers/scores and/or elastography is less accurate and needs to be confirmed by liver biopsy, according to the clinical context (B2[2,5])."
- The guidelines observe that due to non-invasive tests' high negative predictive values, they "may be confidently used for first-line risk stratification to exclude severe disease." Still, they state that "There is no consensus on thresholds or strategies for use in clinical practice when trying to avoid liver biopsy. Some data suggest that the combination of elastography and serum markers performs better than either method alone. Importantly, longitudinal data correlating changes in histological severity and in non-invasive measurements are urgently needed."
- For nonalcoholic steatohepatitis (NASH), the guidelines state that "to date, non-invasive tests are not validated for the diagnosis of NASH" and addresses CK-18 as a proposed biomarker.
- For monitoring of NAFLD, the guidelines state that "Monitoring should include routine biochemistry, assessment of comorbidities and non-invasive monitoring of fibrosis" (EASL et al., 2016).

[1]Grade A Evidence Quality- High: Further research is very unlikely to change our confidence in the estimate of effect

[2]Grade B Evidence Quality- Moderate: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.

[3]Grade C Evidence Quality- Low or very low quality: Further research is very likely to have an important impact on our confidence in the estimate of effect and may change the estimate effect. Any estimate of effect is uncertain.

[4] Grade 1 Recommendation- Strong: Factors influencing the strength of the recommendation included the quality of the evidence, presumed patient-important outcomes, and cost.

[5]Grade 2 Recommendation- Weak: Variability in preferences and values, or more uncertainty. Recommendation is made with less certainty, higher cost or resource consumption.

The EASL also released guidelines on management of Hepatitis C. In it, they recommend that "Fibrosis stage must be assessed by non-invasive methods initially, with liver biopsy reserved for cases where there is uncertainty or potential additional aetiologies (A1[1,4])" (grading scale same as the 2016 guideline above). Non-invasive methods include FibroScan, ARFI, Aixplorer, FibroTest, APRI, and FIB-4 (EASL, 2018).

Guidelines for Hepatitis B were also published. In it, EASL remarks that "the diagnostic accuracy of all non-invasive methods is better at excluding than confirming advanced fibrosis or cirrhosis." Non-invasive methods include assessment of serum biomarkers of liver fibrosis (EASL, 2017).

The EASL also published guidelines titled "Non-invasive tests for evaluation of liver disease severity and prognosis." In it, they state the following (grading scale same as the 2016 guideline above):

- "Serum biomarkers can be used in clinical practice due to their high applicability (>95%) and good interlaboratory reproducibility. However, they should be preferably obtained in fasting patients (particularly those including hyaluronic acid) and following the manufacturer's recommendations for the patented tests (A1[1,4])"

- "Serum biomarkers of fibrosis are well validated in patients with chronic viral hepatitis (with more evidence for HCV than for HBV and HIV/HCV coinfection). They are less well validated in NAFLD and not validated in other chronic liver diseases (A1[1,4])"
- "Their performances are better for detecting cirrhosis than significant fibrosis (A1[1,4])"
- "FibroTest®, APRI and NAFLD fibrosis score are the most widely used and validated patented and nonpatented tests (A1[1,4])"
- "Among the different available strategies, algorithms combining TE and serum biomarkers appear to be the most attractive and validated one (A2[1,5])"
- "HCV patients who were diagnosed with cirrhosis based on non-invasive diagnosis should undergo screening for HCC and PH and do not need confirmatory liver biopsy (A1[1,4])"
- "Non-invasive assessment including serum biomarkers or TE can be used as first line procedure for the identification of patients at low risk of severe fibrosis/ cirrhosis (A1[1,4])"
- "The identification of significant fibrosis is less accurate with non-invasive tests as compared to liver biopsy and may necessitate, according to the clinical context, histological confirmation (A1[1,4])"
- "Follow-up assessment by either serum biomarkers or TE for progression of liver fibrosis should be performed among NAFLD patients at a 3 year interval (B1[2,4])" (EASL & ALEH, 2015).

[1]Grade A Evidence Quality- High: Further research is very unlikely to change our confidence in the estimate of effect
[2]Grade B Evidence Quality- Moderate: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.
[3]Grade 1 Recommendation- Strong: Factors influencing the strength of the recommendation included the quality of the evidence, presumed patient-important outcomes, and cost.
[4]Grade 2 Recommendation- Weak: Variability in preferences and values, or more uncertainty. Recommendation is made with less certainty, higher cost or resource consumption.

The EASL released guidelines on non-invasive tests for evaluation of liver disease severity and prognosis (EASL, 2020). The following recommendations were made (grading scale same as the 2016 guideline above):

- "Serum biomarkers can be used in clinical practice due to their high applicability (>95%) and good interlaboratory reproducibility. However, they should be preferably obtained in fasting patients (particularly those including hyaluronic acid) and following the manufacturer's recommendations for the patented tests (A1[1,4])"
- "TE and serum biomarkers have equivalent performance for detecting significant fibrosis in patients with untreated viral hepatitis (A1[1,4])"
- "In patients with viral hepatitis C, when TE and serum biomarkers results are in accordance, the diagnostic accuracy is increased for detecting significant fibrosis but not for cirrhosis. In cases of unexplained discordance, a liver biopsy should be performed if the results would change the patient management (A1[1,4])"
  "All HCV patients should be screened to exclude cirrhosis by TE if available. Serum biomarkers can be used in the absence of TE (A1[1,4])"(EASL, 2020).

In the 2021 update of the guidelines on non-invasive tests for evaluation of liver disease severity and prognosis (EASL, 2021), the EASL recommends the following for the general population:

- "Non-invasive fibrosis tests should be used for ruling out rather than diagnosing advanced fibrosis in low-prevalence populations (LoE 1, Strong recommendation).

- Non-invasive fibrosis tests should be preferentially used in patients at risk of advanced liver fibrosis (such as patients with metabolic risk factors and/or harmful use of alcohol) and not in unselected general populations (LoE 2, Strong recommendation).
- ALT, AST and platelet count should be part of the routine investigations in primary care in patients with suspected liver disease, so that simple non-invasive scores can be readily calculated (LoE 2, Strong recommendation).
- The automatic calculation and systematic reporting of simple non-invasive fibrosis tests such as FIB-4, in populations at risk of liver fibrosis (individuals with metabolic risk factors and/or harmful use of alcohol) in primary care, is recommended in order to improve risk stratification and linkage to care (LoE 2, Strong recommendation)."

The EASL recommends the following for the diagnosis of compensated advanced chronic liver disease (cACLD) and portal hypertension:

- "cACLD should be diagnosed using second line tests (patented serum tests or elastography) in a specialised setting (LoE 2, strong recommendation).
- Fibrotest® or FibroMeter™ or ELF™ should be used to rule out cACLD if available (LoE 3, strong recommendation).
- LSM by TE should be used to rule-out and diagnose cACLD using the following cut-offs: <8-10 kPa to rule-out; >12-15 kPa to rule-in. Intermediate values require further testing (LoE 3 strong recommendation).
- pSWE and 2D-SWE should be used to rule-out and diagnose cACLD, with AUROCs >0.90 in the published meta-analyses (LoE 2, strong recommendation).
- Inter-system variability should be taken into account when interpreting the results of different elastography techniques, since values, ranges and cut-offs are not comparable (LoE 3, strong recommendation)" (EASL, 2021).

Centers for Disease Control and Prevention (CDC)

The CDC recommends that clinicians offer "medical evaluation (by either a primary care clinician or specialist for chronic liver disease, including treatment and monitoring)" to people who are diagnosed with HCV infection (CDC, 2023).

Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
81517	Liver disease, analysis of 3 biomarkers (hyaluronic acid [HA], procollagen III amino terminal peptide [PIIINP], tissue inhibitor of metalloproteinase 1 [TIMP-1]), using immunoassays, utilizing serum, prognostic algorithm reported as a risk score and risk of liver fibrosis and liver-related clinical events within 5 years
81596	Infectious disease, chronic hepatitis c virus (HCV) infection, six biochemical assays (ALT, A2-macroglobulin, apolipoprotein A-1, total bilirubin, GGT, and haptoglobin) utilizing serum, prognostic algorithm reported as scores for fibrosis and necroinflammatory activity in liver Proprietary test: HCV FibroSURE™, FibroTest™ Laboratory/Manufacturer: BioPredictive S.A.S
81599	Unlisted multianalyte assay with algorithmic analysis
84999	Unlisted chemistry procedure
88341	Immunohistochemistry or immunocytochemistry, per specimen; each additional single antibody stain procedure (List separately in addition to code for primary procedure)
88342	Immunohistochemistry or immunocytochemistry, per specimen; initial single antibody stain procedure
0002M	Liver disease, ten biochemical assays (ALT, A2-macroglobulin, apolipoprotein A-1, total bilirubin, GGT, haptoglobin, AST, glucose, total cholesterol and triglycerides) utilizing serum, prognostic algorithm reported as quantitative scores for fibrosis, steatosis and alcoholic steatohepatitis (ASH) Proprietary test: ASH FibroSURE™ Laboratory/Manufacturer: BioPredictive S.A.S
0003M	Liver disease, ten biochemical assays (ALT, A2-macroglobulin, apolipoprotein A-1, total bilirubin, GGT, haptoglobin, AST, glucose, total cholesterol and triglycerides) utilizing serum, prognostic algorithm reported as quantitative scores for fibrosis, steatosis and nonalcoholic steatohepatitis (NASH) Proprietary test: NASH FibroSURE™ Laboratory/Manufacturer: BioPredictive S.A.S
0166U	Liver disease, 10 biochemical assays (α2-macroglobulin, haptoglobin, apolipoprotein A1, bilirubin, GGT, ALT, AST, triglycerides, cholesterol, fasting glucose) and biometric and demographic data, utilizing serum, algorithm reported as scores for fibrosis, necroinflammatory activity, and steatosis with a summary interpretation Proprietary test: LiverFASt™ Lab/Manufacturer: Fibronostics
0344U	Hepatology (nonalcoholic fatty liver disease [NAFLD]), semiquantitative evaluation of 28 lipid markers by liquid chromatography with tandem mass spectrometry (LC-MS/MS), serum, reported as at-risk for nonalcoholic steatohepatitis (NASH) or not NASH Proprietary test: OWLiver® Lab/Manufacturer: CIMA Sciences, LLC
0468U	Hepatology (nonalcoholic steatohepatitis [NASH]), miR-34a5p, alpha 2-macroglobulin, YKL40, HbA1c, serum and whole blood, algorithm reported as a single score for NASH activity and fibrosis Proprietary test: NASHnextTM (NIS4TM) Lab/Manufacturer: Labcorp

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

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Revision History

Revision Date	Summary of Changes
09/04/2024	Reviewed and Updated: Updated background, guidelines, and evidence-based scientific references. Literature review necessitated the following changes in coverage criteria: Addition of "once every 6 months" to CC1. Updated name of NAFLD and NASH. Now reads: "1) For individuals with hepatitis C, hepatitis B, metabolic dysfunction-associated steatotic liver disease (MASLD) (including metabolic dysfunction-associated steatohepatitis [MASH]), or alcoholic hepatitis, the use of the following multianalyte assays with algorithmic analysis to distinguish hepatic cirrhosis from non-cirrhosis MEETS COVERAGE CRITERIA once every 6 months:" CC2 updated name of NAFLD to MASLD.
06/19/2024	Off-cycle coding modification: Added CPT code 0468U (effective date 07/01/2024)
12/06/2023	Off-cycle coding modification: Added CPT code 81517. New code effective 1/1/2024. Removed CPT code 0014M. Code deleted effective 12/31/2023.

Serum Tumor Markers for Malignancies

Policy Number: AHS – G2124 – Serum Tumor Markers for Malignancies	Prior Policy Name and Number, as applicable:
Initial Presentation Date: 09/18/2015 Effective Date: February 1, 2025	

Policy Description

Circulating tumor biomarkers are substances detected in the blood, urine, or other body fluids that are either produced by a tumor itself or in response to its presence. These biomarkers can be used to help detect, diagnose, stage, and manage some types of cancer, because their amounts are typically elevated in individuals harboring a tumor (Hottinger & Hormigo, 2011; NCI, 2023). There are currently dozens of tumor markers in common use; this laboratory policy addresses tumor markers which may be measured in an individual's serum.

Terms such as male and female are used when necessary to refer to sex assigned at birth.

The following management of serum tumor markers is built from recommendations from the National Comprehensive Cancer Network (NCCN) Biomarkers Compendium®, which contains information "designed to support decision making around the use of biomarker testing in patients with cancer. The NCCN Biomarkers Compendium® is updated in conjunction with the NCCN Guidelines on a continual basis" (NCCN, 2023).

Related Policies

Policy Number	Policy Title
	Not applicable

Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

Note: Except for where otherwise specified in the coverage criteria below, quarterly measurement of designated serum tumor markers is permitted for follow-up, monitoring, and/or surveillance

1) Measurement of the following serum tumor markers **MEETS COVERAGE CRITERIA** for the following indications:

Serum Tumor Marker	Indication
Alkaline phosphatase (ALP)	Bone neoplasms: **workup**; **during treatment**; **surveillance**
	Systemic light chain amyloidosis: **initial diagnostic workup**
Alpha fetoprotein (AFP)	Hepatocellular carcinoma: **screening**; **workup** for confirmed HCC; **surveillance** (every 3-6 months for 2 years, then every 6 months)
	Intrahepatic cholangiocarcinoma: **workup** for isolated intrahepatic mass
	Occult primary: **additional workup** for localized adenocarcinoma or carcinoma not otherwise specified; liver, mediastinum, or retroperitoneal mass
	Ovarian cancer/fallopian tube cancer/primary peritoneal cancer: **initial workup**; **during primary chemotherapy**; **monitoring/follow-up for complete response** (as clinically indicated)
	Ovarian cancers (less common): – Carcinosarcoma (malignant mixed mullerian tumors): **monitoring/follow-up** – Clear cell carcinoma of the ovary: **monitoring/follow-up** – Grade 1 endometrioid carcinoma: **monitoring/follow-up** – Mucinous neoplasms of the ovary: **monitoring/follow-up** – Low-grade serous carcinoma: **monitoring/follow-up**
	Ovarian cancers: – Borderline epithelial tumors: **monitoring/follow-up** (every visit if initially elevated) – Malignant germ cell tumors: **surveillance** (no more than every 2 months for the first 2 years, every 4 months in years 3-5, and then annually after year 5) – Malignant sex cord stromal tumors: **surveillance** if clinically indicated. If done, frequency based on stage (i.e., 6-12 months if early-stage, low-risk disease; 4-6 months if high-risk disease)
	Testicular cancer – nonseminoma: **post-diagnostic workup**; **risk classification**; **surveillance** (no more than every 2 months)
	Testicular cancer - pure seminoma: **initial diagnostic workup**; **post-diagnostic workup**; **risk classification**; **post-treatment surveillance** (no more than every 2 months)
	Thymomas and thymic carcinomas: **initial evaluation**, if appropriate

Serum Tumor Marker	Indication
Beta-2 microglobulin (B2M)	B-cell lymphomas (Castleman disease; diffuse large B-cell; follicular [grade 1-2]; HIV-related; lymphoblastic; mantle cell): **workup**
	Chronic lymphocytic leukemia/small lymphocytic lymphoma: **workup**; for **prognostic** and/or **therapy determination**
	Multiple myeloma: **initial diagnostic workup**; **follow-up/surveillance** (as needed) for solitary plasmacytoma or solitary plasmacytoma with minimal marrow involvement
	Systemic light chain amyloidosis: **initial diagnostic workup**
	Waldenström macroglobulinemia / lymphoplasmacytic lymphoma: **workup**
Beta human chorionic gonadotropin (beta-HCG)	Gestational trophoblastic neoplasia: **initial workup**; **during and post treatment** (no more than weekly); **follow-up/surveillance** (no more than monthly for 12 months)
	Occult primary: **additional workup** for localized adenocarcinoma or carcinoma not otherwise specified; individuals < 65 years of age with testes presenting with retroperitoneal mass
	Ovarian cancer/fallopian tube cancer/primary peritoneal cancer: **initial workup**; **during primary chemotherapy**; **monitoring/follow-up for complete response** (as clinically indicated)
	Ovarian cancers: – Borderline epithelial tumors: **monitoring/follow-up** (every visit if initially elevated) – Malignant germ cell tumors: **surveillance** (no more than every 2 months for the first 2 years, every 4 months in years 3-5, and then annually after year 5) – Malignant sex cord stromal tumors: **surveillance** if clinically indicated. If done, frequency based on stage (i.e., 6-12 months if early-stage, low-risk disease; 4-6 months if high-risk disease)
	Testicular cancer – nonseminoma: **post-diagnostic workup**; **risk classification**; **surveillance** (no more than every 2 months)
	Testicular cancer - pure seminoma: **initial diagnostic workup**; **post-diagnostic workup**; **risk classification**; **post-treatment surveillance** (no more than every 2 months)
	Thymomas and thymic carcinomas: **initial evaluation**, if appropriate
BNP or NT-proBNP	Multiple myeloma: **initial diagnostic workup**
	Systemic light chain amyloidosis: **initial diagnostic workup**
Calcitonin (CALCA)	Adenocarcinoma, and anaplastic/undifferentiated epithelial tumors: **workup**
	Medullary carcinoma: **additional workup**; **post-surgical evaluation**; **monitoring**; **surveillance** (2-3 months postoperative, then every 6-12 months)
	Multiple endocrine neoplasia, type 2: **at diagnosis** (clinical evaluation) for medullary thyroid cancer
	Occult primary (unknown primary cancer): **workup**
Cancer antigen 15-3 and 27.29	Breast cancer (invasive): **monitoring** metastatic disease

Serum Tumor Marker	Indication
(CA 15-3 and 27.29)	Occult primary: suspected metastatic malignancy: **initial workup**; assessing disease **prognosis**; **monitoring/follow-up** for response
Cancer antigen 19-9 (CA 19-9)	Ampullary adenocarcinoma: **workup**; **surveillance** (every 3-6 months for 2 years, then every 6-12 months for up to 5 years as clinically indicated) for resected ampullary cancer, stage I-III
	Appendiceal adenocarcinoma: **workup** to establish baseline. Abnormal measurements should be trended
	Extrahepatic cholangiocarcinoma: **workup** to establish baseline; **monitoring**
	Gallbladder cancer: **workup** to establish baseline; **monitoring**; **surveillance** (as clinically indicated), post-resection
	Intrahepatic cholangiocarcinoma: **workup** to establish baseline; **monitoring**
	Occult primary: workup to establish baseline; assessing disease **prognosis**; **monitoring/follow-up** for response
	Ovarian cancer/fallopian tube cancer/primary peritoneal cancer: **initial workup**; **during primary chemotherapy**; **monitoring/follow-up for complete response** (as clinically indicated)
	Ovarian cancers (less common): – Carcinosarcoma (malignant mixed mullerian tumors): **workup** – Clear cell carcinoma of the ovary: **workup** – Grade 1 endometrioid carcinoma: **workup** – Low-grade serous carcinoma: **workup** – Mucinous neoplasms of the ovary: **workup**
	Ovarian cancers – Borderline epithelial tumors: **monitoring/follow-up** (every visit if initially elevated) – Malignant germ cell tumors: **surveillance** (no more than every 2 months for the first 2 years, every 4 months in years 3-5, and then annually after year 5) – Malignant sex cord stromal tumors: **surveillance** if clinically indicated. If done, frequency based on stage (i.e., 6-12 months if early-stage, low-risk disease; 4-6 months if high-risk disease) – Mucinous carcinoma of the ovary: **additional workup** (if not previously done)
	Pancreatic adenocarcinoma: **workup** to establish baseline; **monitoring**; **post-operative**, **post-adjuvant treatment surveillance** (every 3-6 months for 2 years, then every 6-12 months as clinically indicated)
	Small bowel adenocarcinoma: **workup** to establish baseline; **post-treatment surveillance** (every 3-6 months for 2 years, then every 6 months for a total of 5 years); **at metastasis or recurrence**
Cancer antigen 125 (CA-125)	Appendiceal adenocarcinoma: **workup** to establish baseline
	Endometrial carcinoma: **additional workup**; **surveillance** (if initially elevated)
	Lynch syndrome: **surveillance**

Serum Tumor Marker	Indication
	Occult primary: **additional workup** for adenocarcinoma or carcinoma not otherwise specified, in those with a uterus and/or ovaries present
	Ovarian cancer/fallopian tube cancer/primary peritoneal cancer: **initial workup**; **during primary chemotherapy**; **monitoring/follow-up for complete response** (as clinically indicated)
	Ovarian cancers (less common): – Carcinosarcoma (malignant mixed mullerian tumors): **monitoring/follow-up** – Clear cell carcinoma of the ovary: **monitoring/follow-up** – Mucious neoplasms of the ovary: **monitoring/follow-up** – Grade 1 endometrioid carcinoma: **monitoring/follow-up** – Low-grade serous carcinoma: **monitoring/follow-up**
	Ovarian cancers: – Borderline epithelial tumors: **monitoring/follow-up** (every visit if initially elevated) – Malignant germ cell tumors: **surveillance** (no more than every 2 months for the first 2 years, every 4 months in years 3-5, and then annually after year 5) – Malignant sex cord stromal tumors: **surveillance** if clinically indicated. If done, frequency based on stage (i.e., 6-12 months if early-stage, low-risk disease; 4-6 months if high-risk disease)
	Peritoneal mesothelioma: **initial evaluation**
	Uterine neoplasms: **initial workup**
Carcinoembryonic antigen (CEA)	Appendiceal adenocarcinoma: **workup** to establish baseline; **monitoring**; **post-treatment surveillance**
	Breast cancer (invasive): **Monitoring** metastatic disease
	Colon cancer: **workup** to establish baseline; **monitoring**; **surveillance** (every 3-6 months for 2 years, then every 6 months for a total of 5 years)
	Extrahepatic cholangiocarcinoma: **workup** to establish baseline; **monitoring**
	Gallbladder cancer: **workup** to establish baseline; **monitoring**; **surveillance; monitoring of adjuvant treatment** (as clinically indicated), **post-resection**
	Intrahepatic cholangiocarcinoma: **workup** to establish baseline; **monitoring**
	Medullary carcinoma: **diagnosis and additional workup**; **monitoring**; **post-surgical surveillance** (2-3 months postoperative, then every 6-12 months)
	Multiple endocrine neoplasia, type 2: **at diagnosis** (clinical evaluation) for medullary thyroid cancer
	Ovarian cancer/fallopian tube cancer/primary peritoneal cancer: **initial workup**; **during primary chemotherapy**; **monitoring/follow-up for complete response** (as clinically indicated)
	Ovarian cancers (less common): – Carcinosarcoma (malignant mixed mullerian tumors: **monitoring/follow-up** – Clear cell carcinoma of the ovary: **monitoring/follow-up**

Serum Tumor Marker	Indication
	– Grade 1 endometrioid carcinoma: **monitoring/follow-up** – Low-grade serous carcinoma: **monitoring/follow-up** – Mucinous neoplasms of the ovary: **monitoring/follow-up**
	Ovarian cancers : – Borderline epithelial tumors: **monitoring/follow-up** (every visit if initially elevated); **post-adjuvant treatment** – Malignant germ cell tumors: **surveillance** (no more than every 2 months for the first 2 years, every 4 months in years 3-5, and then annually after year 5) – Malignant sex cord stromal tumors: **surveillance** if clinically indicated. If done, frequency based on stage (i.e., 6-12 months if early-stage, low-risk disease; 4-6 months if high-risk disease) – Mucinous carcinoma of the ovary: **additional workup** (if not previously done)
	Rectal cancer: **workup** to establish baseline; **monitoring**; **surveillance** (every 3-6 months for 2 years, then every 6 months for a total of 5 years)
	Small bowel adenocarcinoma: **workup** to establish baseline; **post-treatment surveillance** (every 3-6 months for 2 years, then every 6 months for a total of 5 years)
Inhibin (INHA)	Adrenocortical carcinoma: **workup**
	Ovarian cancer/fallopian tube cancer/primary peritoneal cancer: **initial workup**; **during primary chemotherapy**; **monitoring/follow-up for complete response** (as clinically indicated)
	Ovarian cancers (less common): – Carcinosarcoma (malignant mixed mullerian tumors: **monitoring/follow-up** – Clear cell carcinoma of the ovary: **monitoring/follow-up** – Grade 1 endometrioid carcinoma: **monitoring/follow-up** – Low-grade serous carcinoma: **monitoring/follow-up** – Mucinous neoplasms of the ovary: **monitoring/follow-up**
	Ovarian cancers: – Borderline epithelial tumors: **monitoring/follow-up** (every visit if initially elevated) – Malignant Germ cell tumors: **surveillance** (no more than every 2 months for the first 2 years, every 4 months in years 3-5, and then annually after year 5) – Malignant sex cord stromal tumors: **surveillance** if clinically indicated. If done, frequency based on stage (i.e., 6-12 months if early-stage, low-risk disease; 4-6 months if high-risk disease)
Lactate dehydrogenase (LDH)	B-cell lymphomas (Burkitt; Castleman disease; diffuse large B-cell; extranodal marginal zone lymphoma of nongastric sites [noncutaneous] and of the stomach; follicular [grade 1-2]; HIV-related; lymphoblastic; mantle cell; nodal marginal zone;pediatric aggressive mature; post-transplant lymphoproliferative disorders; primary cutaneous; splenic marginal zone): **workup**
	Bone neoplasms: **workup**

Serum Tumor Marker	Indication
	Chronic lymphocytic leukemia/small lymphocytic lymphoma: **workup**, and at transformation or histologic progression (if applicable)
	Hairy cell leukemia: **workup**
	Kidney cancer: **initial workup**
	Melanoma (cutaneous and uveal): **workup** for metastatic or recurrent disease
	Multiple myeloma: **initial workup**; **surveillance** (as needed) post primary treatment for solitary plasmacytoma or solitary plasmacytoma with minimal marrow involvement
	Ovarian cancer/fallopian tube cancer/primary peritoneal cancer: **initial workup**; **during primary chemotherapy**, **monitoring/follow-up for complete response** (as clinically indicated)
	Ovarian cancers (less common): – Carcinosarcoma (malignant mixed mullerian tumors: **monitoring/follow-up** – Clear cell carcinoma of the ovary: **monitoring/follow-up** – Grade 1 endometrioid carcinoma: **monitoring/follow-up** – Low-grade serous carcinoma: **monitoring/follow-up** – Mucinous neoplasms of the ovary: **monitoring/follow-up**
	Ovarian cancers: – Borderline epithelial tumors: **monitoring/follow-up** (every visit if initially elevated) – Malignant germ cell tumors: **surveillance** (no more than every 2 months for the first 2 years, every 4 months in years 3-5, and then annually after year 5) – Malignant sex cord stromal tumors: **surveillance** if clinically indicated. If done, frequency based on stage (i.e., 6-12 months if early-stage, low-risk disease; 4-6 months if high-risk disease)
	Primary cutaneous lymphomas (mycosis fungoides/Sezary syndrome; primary cutaneous CD30+ T-cell lymphoproliferative disorders): **workup**
	Systemic light chain amyloidosis: **initial diagnostic workup**
	Systemic mastocytosis: **initial diagnostic workup**
	T-cell lymphomas (adult T-cell; breast implant-associated ALCL; extranodal NK/T-cell; hepatosplenic; peripheral; T-cell prolymphocytic leukemia): **workup**; **staging** (breast implant-associated ALCL only)
	Testicular cancer – nonseminoma: **post-diagnostic workup**; **risk classification**; **surveillance** (no more than every 2 months)
	Testicular cancer – pure seminoma: **initial diagnostic workup**; **post-diagnostic workup**; **risk classification**; **post-treatment surveillance** (no more than every 2 months)
	Waldenström macroglobulinemia / lymphoplasmacytic lymphoma: **workup**
Serum free light chain	Multiple myeloma: **initial diagnostic workup**; **surveillance** (up to once per month)
	Systemic light chain amyloidosis: **initial diagnostic workup**

Serum Tumor Marker	Indication
Troponin T	Systemic light chain amyloidosis: **initial diagnostic workup**
Tryptase	Systemic mastocytosis: **initial diagnosis**

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

2) For all other cancer indications not discussed above, use of the above biomarkers (alone or in a panel of serum tumor markers) **DOES NOT MEET COVERAGE CRITERIA.**

3) All other serum tumor markers not addressed above (alone or in a panel of serum tumor markers) **DO NOT MEET COVERAGE CRITERIA.**

4) For the screening and detection of cancer, analysis of proteomic patterns in serum **DOES NOT MEET COVERAGE CRITERIA**.

Table of Terminology

Term	Definition
A2-PAG	Pregnancy associated alpha 2 glycoprotein
AACC	American Association for Clinical Chemistry
AASLD	American Association for the Study of Liver Diseases
ACCP	American College of Chest Physicians
ACR	American College of Radiology
ADLM	Association for Diagnostics & Laboratory Medicine
AFP	Alpha fetoprotein
AGA	American Gastroenterological Association
AGCT	Adult-type granulosa cell tumor
AIDS	Acquired immune deficiency syndrome
ALL	Acute lymphoblastic leukemia
ALP	Alkaline phosphatase
AMH	Anti-müllerian hormone
AML	Acute myeloid leukemia
ASCO	American Society of Clinical Oncology
ATA	American Thyroid Association
AUC	Area under curve
B7-H4	V-set domain-containing T-cell activation inhibitor 1
B2M	Beta-2 microglobulin
BCM	Breast cancer mucin
beta-HCG	Beta-human chorionic gonadotropin
BG8	Blood group 8
BNP	Brain natriuretic peptide

BRCA	Breast cancer gene
BRCA1	*Breast cancer gene 1*
BRCA2	*Breast cancer gene 2*
CA	Cancer antigen
CALCA	Calcitonin
CAM 17-1	Antimucin monoclonal antibody
CAM-26	Carcinoma associated mucin antigen
CAM-29	Carcinoma associated mucin antigen
CAR-3	Antigenic determinant recognized by monoclonal antibody AR-3
CA-SCC	Squamous cell carcinoma antigen
CEA	Carcinoembryonic antigen
CEACAM6	Carcinoembryonic antigen cell adhesion molecule 6
CEACAM-7	Carcinoembryonic antigen cellular adhesion molecule-7
CEP17	Chromosome 17 centromere
CFL1	Cofilin
CgA	Chromogranin A
CLIA ’88	Clinical Laboratory Improvement Amendments of 1988
CMS	Centers for Medicare and Medicaid Services
CRC	Colorectal cancer
CSS	Cancer specific survival
CTC	Circulating tumor cell
CUP	Cancers of unknown primary
CYP2D6	*Cytochrome P450 2D6*
DCIS	Ductal carcinoma in situ
DCP	Des-γ-carboxy prothrombin
DcR3	Decoy receptor 3
DFS	Disease-free survival
DMSA	Pentavalent technetium-99mm dimercaptosuccinic
Du-PAN-2	Sialylated carbohydrate antigen
EASL	European Association for the Study of the Liver
ECM	Extracellular matrix protein
EGFR	Epidermal growth factor receptor
ELISA	Enzyme-linked immunosorbent assay
EPCAM	Epithelial cell adhesion molecule
ER	Estrogen receptor
FDA	Food and Drug Administration
FLC	Free-light chain
FOXP3	Forkhead box P3
GC	Gastric cancer
GCTs	Germ cell tumors
GRP78	78-kDa glucose-regulated protein
HCC	Hepatocellular carcinoma
hCGβ	Free β-subunit of human chorionic gonadotropin

HE4	Human epididymis protein 4
HEC1	Highly expressed in cancer protein
HER2	Human epidermal growth factor receptor 2
HR	Hazard ratio
HYAL1	Hyaluronoglucosaminidase
IGF	Insulin-like growth factors
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IHC	Immunohistochemistry
INHA	Inhibin
Ki-67	Antigen KI-67
KRAS	Kirsten rat sarcoma viral oncogene homolog
LCA	Lens culinaris agglutinin
LCOC	Less common ovarian cancers
LCOH	Less common ovarian histopathologies
LDH	Lactate dehydrogenase
LDT	Laboratory-developed test
LINE-1	Long interspersed nuclear elements 1
MALDI	Matrix-assisted laser desorption/ionization
MAP	Microtubule-associated protein
MCA	Mucinous carcinoma associated antigen
MGUS	Monoclonal gammopathy of undetermined significance
MHC	Major histocompatibility complex
MINDACT	Microarray in node-negative disease may avoid chemotherapy
MMP-1	Matrix metalloproteinase-1
mRNA	Messenger ribonucleic acid
MSA	Mammary serum antigen
MTC	Medullary thyroid carcinoma
NACB	National Academy of Clinical Biochemistry
NANETS	North American Neuroendocrine Tumor Society
NCCN	National Comprehensive Cancer Network
NET	Neuroendocrine tumor cells
NICE	National Institute for Health and Clinical Excellence
NMP22	Nuclear matrix protein 22
non-HCC	Non-hepatocellular carcinoma
NSE	Neuron specific enolase
NSGCT	Nonseminomatous germ cell tumor
NT-proBNP	N-terminal pro hormone B-type natriuretic peptide
OS	Overall survival
P53	Tumor protein P53
PAGE	Polyacrylamide gel electrophoresis
PAI-1	Plasminogen activator inhibitor type 1

PAM50-ROR	Prediction analysis of microarray 50-risk of recurrence
PcSt	Pancreastatin
PD-L1	Programmed Death-ligand 1
PED-ALL	Pediatric acute lymphoblastic leukemia
PgR	Plant growth regulator
PIVKA-II	Protein induced by vitamin K absence/antagonist-II
P-LAP	Placental alkaline phosphatase
PNA-ELLA	Peanut lectin bonding assay
PR	Progesterone receptor
PSA	Prostate specific antigen
PTEN	Phosphatase and tensin homolog
RCC	Renal cell carcinoma
RMI I	Risk of malignancy index I
ROC	Receiver operating characteristic
ROMA	Risk of ovarian malignancy algorithm
ROR	Risk of recurrence
RRSO	Risk-reducing salpingo-oopherectomy
SCC	Squamous cell carcinoma
SCLCs	Small cell lung cancers
SLEX	Sialylated lewis-x antigen
SLX	Sialylated SSEA-1 antigen
SPAN-1	Sialylated carbonated antigen span-1
ST-439	Sialylated carbohydrate antigen st-439
STMs	Serum tumor markers
TAG	Tumor associated glycoprotein
TATI	Tumor associated trypsin inhibitor
TILs	Tumor-infiltrating lymphocytes
TIMP-1	Tissue inhibitor of metalloproteinase-1
TKI	Tyrosine kinase inhibitor
TN	Triple-negative
TNF-a	Tumor necrosis factor alpha
TnI	Troponin I
TnT	Troponin T
TOP2A	Deoxyribonucleic acid topoisomerase II alpha
TPA	Tissue polypeptide antigen
TPS	Tissue polypeptide specific antigen
TTF-1	Thyroid transcription factor-1
TVUS	Transvaginal ultrasound
uPA	Urokinase plasminogen activator
uPAR	Urokinase plasminogen activator receptor
WM	Waldenström's Macroglobulinemia
WT1	Wilms' tumor protein

Scientific Background

Actionable molecular assays for tumor biomarkers may guide treatment decisions for common malignancies (Febbo et al., 2011). Circulating tumor biomarkers are proteins detected in blood, urine, or other body fluids that serve as surrogate indicators to increase or decrease the clinician's suspicion of future clinically important events. These can be used to determine risk, screen for early cancers, establish diagnosis, estimate prognosis, predict that a specific therapy will work, and/or monitor for disease recurrence or progression (Catharine M. Sturgeon et al., 2008). The National Comprehensive Cancer Network (NCCN) task force guidelines recommend that tumor markers be classified by indication as diagnostic, prognostic, predictive, and companion tests. An individual marker may serve more than one purpose and thus can fall into more than one category of biomarker. Biomarkers may also have different categorization across different stages of disease or different types of tumors (Febbo et al., 2011). Some of these categories are listed below:

- **Diagnostic** – Tumor biomarkers that aid in the diagnosis or subclassification of a particular disease state. Detection of diagnostic biomarkers may result in different management of the disease, but the marker is used primarily to establish that a particular disease is present in the patient sample. An example of a diagnostic biomarker is the Philadelphia chromosome in chronic myelogenous leukemia.
- **Prognostic** – Some tumor biomarkers have an association with certain clinical outcomes, such as overall survival or recurrence-free survival, independent of the treatment rendered. An example is a mutant p53 gene, whose presence may indicate a more aggressive type of cancer.
- **Predictive** – Tumor biomarkers can predict the activity of a specific class or type of therapy and are used to help make more specific treatment decisions. An example is human epidermal growth factor 2 (HER2), which is assessed in breast cancer patients. Patients who are negative for this biomarker do not respond as well to trastuzumab.
- **Companion** – Biomarkers may be diagnostic, prognostic, or predictive, but are used to identify a subgroup of patients for whom a therapy has shown benefit. This category of biomarker is similar to the predictive category, but these biomarkers do not usually have independent prognostic or predictive strength (Febbo et al., 2011).

Proprietary Testing

There are laboratory developed tests that utilize serum tumor markers intended to aid in the management of individuals with cancer or those at increased risk of developing cancer. The clinical validity and utility of these tests is still emerging. Examples of commercialized tests in current use include the following:

BeScreened™–CRC is a colorectal cancer (CRC) screening test. BeScreened™–CRC tests three blood-based proteins that are thought to play a role in the immunological activities of colorectal cancer. The test results are reported as either "negative" or "positive" for the likely presence of CRC. The test is reported to have 94% accuracy in determining the "likely presence or absence of colorectal cancer." The test developer reports "BeScreened™-CRC is not a test for colorectal cancer diagnosis; it is a screening test that aides in the detection of colorectal cancer and is not intended to replace a colonoscopy" (BeScreened, 2024).

REVEAL Lung Nodule Characterization is a blood test that aids in "characterizing indeterminate pulmonary nodules (4-30mm) in current smokers aged 25 years and older." The test results are based on three clinical factors and three blood proteins associated with lung cancer. "REVEAL Lung Nodule Characterization is a risk assessment tool, that is to be used only in conjunction with standard clinical assessments. The test is not intended as a screening or stand-alone diagnostic assay" (MagArray, 2024).

Ova1® and Overa® are blood tests for ovarian cancer risk assessment that both have FDA clearance for women with pelvic masses who are planned for surgery. Each test measures five ovarian cancer-associated markers and contributes differently to the overall risk assessment analysis: Ova1® is performed first to determine an initial risk; if the result is indeterminate, Overa® will be automatically performed to in attempt to refine the initial result (ASPIRA, 2024a).

For individuals with an adnexal mass who are not planned for surgery, OvaWatch℠ may be considered for ovarian cancer risk refinement when initial assessment of the mass was indeterminate or benign. This test considers seven tumor biomarkers, an individual's age, and menopausal status, to produce a single risk assessment score with a reported negative predictive value of 99% (ASPIRA).

Clinical Utility and Validity

Most biomarkers are not specific for tumors or organs and their levels may rise in other diseases. The diagnostic value of a tumor marker will depend on the prevalence of the disease and on the specificity and sensitivity of the marker (Hottinger & Hormigo, 2011). The analytic and clinical validity as well as the clinical utility of each biomarker should be taken into account before it is used for screening and or management of malignancies (Catharine M. Sturgeon et al., 2008). Establishing a biomarker's ability to associate with a given outcome of interest (diagnostic, prognostic, et al.) and ability to improve clinical outcomes and decision making is critical (Febbo et al., 2011).

With respect to biomarker acquisition, growing evidence continues to support the utility of liquid biopsy. Compared to the "gold standard" tissue biopsy, serum can be obtained in a relatively non-invasive manner, without the need for surgery and the associated risks and recovery time. Further, serum is generally always available; tumor tissue, conversely, may not always be accessible or present in a clinically useful quantity (Pinzani et al., 2021).

Alkaline phosphatase (ALP)

Alkaline phosphatase is an enzyme that is highly concentrated in the liver, kidneys, placenta, and bone (Sharma et al., 2014). While the physiological functions of the various isozymes of ALP are incompletely understood, there is a stronger consensus that the bone isoenzyme contributes to skeletal mineralization (Szulc et al., 2013). Serum ALP has thus been identified as a useful marker for diseases of the bone and liver, and is often measured during the workup and management of disorders that include bone neoplasms, systemic light chain amyloidosis to confirm liver involvement, as well as other cancerous and non-cancerous conditions (NCCN, 2024c; Sharma et al., 2014; Thio et al., 2020).

Alpha-fetoprotein (AFP)

Alpha-fetoprotein is a commonly assessed biomarker in cancer patients. AFP is a protein that is normally produced by the fetal yolk sac, and its concentration stabilizes at approximately < 10 µg/L shortly after

birth (Schefer et al., 1998). Many tissues produce this protein if they become malignant, and AFP is elevated in a variety of cancers, such as hepatocellular carcinomas (HCC). False positives may occur due to liver damage or a rare hereditary syndrome (Gilligan et al., 2010).

Alpha-fetoprotein can be fractionated into three different isoforms based on reactivity with Lens culinaris agglutinin (LCA), and the three types are as follows: L1 (no reactivity), L2 (low reactivity), and L3 (high reactivity). AFP-L3 is theorized to associate with HCC because the dedifferentiation of HCC tissues correlates with the production of the enzyme that produces AFP-L3. This means that AFP-L3 may be closely related to cancer-specific events and are at least more specific to certain malignant cancers (M. Wu et al., 2018).

A study by Santos Schraiber et al. (2016) assessed the ability to predict recurrence of HCC after liver transplant using AFP. The authors analyzed 206 patients and the recurrence frequency was found to be 15.5%. However, the authors' multivariate analysis found that the only risk factor for recurrence was an AFP level of >200 ng/mL, which was associated with a 3.32 times higher increase in the probably of HCC recurrence. The authors noted that recurrence was also associated with lower survival rate (Santos Schraiber et al., 2016).

Cheng et al. (2014) conducted a meta-analysis of fifteen studies (4465 patients) to evaluate the association of high pre-treatment serum AFP-L3 percentage (%) with overall survival (OS) and disease-free survival (DFS) in HCC patients. The authors found that high pre-treatment serum AFP-L3% implied poor OS (Hazard Ratio [HR]: 1.65), and DFS (HR: 1.80) of individuals with HCC. The authors found an association between pre-treatment serum AFP-L3% and OS and DFS in low AFP concentration HCC patients (HR: 1.96 and 2.53 respectively). The authors concluded that "high pre-treatment serum AFP-L3% levels indicated a poor prognosis for patients with HCC" (Cheng et al., 2014).

Park et al. (2017) compared the diagnostic values of AFP, AFP-L3, and protein induced by vitamin K absence/antagonist-II (PIVKA-II) individually and in combination to find the best biomarker or biomarker panel. A total of 79 patients with newly diagnosed HCC and 77 control patients with liver cirrhosis were enrolled. When the three biomarkers were analyzed individually, AFP showed the largest area under the receiver-operating characteristic curve (AUC) (0.751). For combinations of the biomarkers, the AUC was highest (0.765) for PIVKA-II > 40 mAU/mL and AFP > 10 ng/mL. Adding AFP-L3 > 10% led to worse sensitivity and lower AUC. The authors concluded that "the diagnostic value of AFP was improved by combining it with PIVKA-II, but adding AFP-L3 did not contribute to the ability to distinguish between HCC and non-HCC liver cirrhosis" and that "AFP showed the best diagnostic performance as a single biomarker for HCC" (Park et al., 2017).

Ryu et al. (2017) investigated the prognostic implications of the expression patterns of three tumor markers, AFP, AFP-L3, and des-γ-carboxy prothrombin (DCP). The study included 1182 consecutive patients that underwent hepatic resection and surgical microwave ablation for HCC. This study analyzed 475 patients within the Milan criteria and Child-Pugh class A. Cumulative OS and DFS rates were analyzed relative to the number of positive tumor markers. OS and DFS at five years postoperatively were 85.3 and 44.2% in triple-negative patients, 79.4 and 48.0% in single-positive patients, 56.2 and 32.9% in double-positive patients, and 61.7 and 35.7% in triple-positive patients. The authors concluded that "both double- and triple-positive tumor markers are associated with early recurrence and poor survival in HCC patients within the Milan criteria and Child-Pugh class A" (Ryu et al., 2017).

Caviglia et al. (2016) conducted a study evaluating AFP, AFP-L3, and DCP as detection tools for HCC. A total of 98 patients were enrolled (44 without HCC, 54 with), and the FDA-approved automated immunoassay system uTASWako was used to measure these biomarkers. AFP-L3 demonstrated an AUC of 0.867, a sensitivity of 0.849, a specificity of 0.886, a negative predictive value of 0.830, and a positive predictive value of 0.900. The combination of all three biomarkers had an accuracy of 87.6%. The overall accuracy of uTASWako was 84.5%. The authors concluded that the uTASWako had a "high analytical performance" and that the biomarker combination was superior to any of the individual markers alone (Caviglia et al., 2016).

Beta-2 microglobulin (B2M)

Beta-2 microglobulin is the light chain component of the MHC-1 molecule and is present in most cells of the body (Berrebi et al., 2009). This protein may aggregate and eventually form insoluble amyloid fibrils, which cause numerous conditions such as bone and joint damage (Katou et al., 2002; Marcinko et al., 2017). Elevated serum levels of B2M have been associated with cancers such as multiple myeloma or chronic leukocytic leukemia (Berrebi et al., 2009).

Seo et al. (2016) examined the prognostic value of B2M for diffuse large B-cell lymphoma. A total of 833 patients at a ≥2.5 mg/L cutoff were analyzed, and both five-year survival and overall survival rates were found to be significantly worse in patients with elevated B2M (290 patients or 34.8%). The elevated B2M cohort was calculated to have a 41% five-year survival rate and a 49.2% overall survival rate, compared to 76.1% five-year survival and 83.8% overall survival for the remaining 543 patients (Seo et al., 2016).

Beta-human chorionic gonadotropin (beta-hCG)

Beta-human chorionic gonadotropin is the beta subunit of the normal hCG hormone produced during pregnancy. Some malignancies express the gene for the beta subunit of hCG, thereby producing this protein independent of pregnancy (Harvey, 2023). The beta subunit is responsible for providing the biological and immunological specificity to each hormone (Marcillac et al., 1992). This biomarker is typically associated with aggressive disease in nontrophoblastic tumors. This biomarker may be elevated in ovarian cancers, testicular cancers, and more (Hotakainen et al., 2002).

Li et al. (2018) evaluated beta-hCG as a marker for CRC. In total, 50 patients out of 136 patients expressed beta-hCG at the "invasive front." The authors found higher expression of beta-hCG to be associated with worse prognosis than those with low beta-hCG expression and reported that beta-hCG "promoted the migration and invasion of CRC in vitro and in vivo but had no effect on the proliferation of tumor cells." A correlation was also found between beta-HCG expression level and tumor invasion in early-stage CRC patients (Li et al., 2018).

BNP/NT-proBNP

Brain natriuretic peptide (also known as B-type natriuretic peptide) is thought to play important roles in the regulation of blood pressure, blood volume, and sodium balance (Di Castelnuovo et al., 2019; Weber & Hamm, 2006). BNP is synthesized as a prehormone (proBNP) within cardiomyocytes that is cleaved into the biologically active 32 amino acid BNP and the inactive 76 amino acid N-terminal fragment (NT-proBNP) (Weber & Hamm, 2006).

Interest in BNP as a potential marker for cardiac function has existed for decades, lending credence to the utility of BNP to aid in the management of disorders that may affect the heart. These include systemic light chain amyloidosis and multiple myeloma, where serum concentrations of BNP or NT-proBNP may inform the degree of heart involvement (NCCN, 2024b, 2024c; Venner, 2019).

Calcitonin

Serum calcitonin is the primary tumor marker for medullary thyroid carcinoma (MTC). MTC is a neuroendocrine tumor of the parafollicular or C cells of the thyroid gland, and production of calcitonin is a signifying characteristic of this tumor. The concentration of calcitonin tends to correlate with tumor mass (Tuttle, 2022). However, the American Thyroid Association (ATA) has noted that there is a lack of agreement on the utility of routine calcitonin measurement as a screening test for individuals with thyroid nodules (Haugen et al., 2016; Wells et al., 2015).

Tormey et al. (2017) evaluated measurement of serum calcitonin in patients presenting with thyroid nodules. A total of 44 patients were evaluated and 33 of the patients were reported to not have "detectable serum calcitonin," noting that three patients had an initially elevated serum concentration that became undetectable. The authors also note that out of the 2070 patients in their sample, only seven cases of MTC were diagnosed. The authors recommended not screening routinely for MTC (Tormey et al., 2017).

Cancer antigens (CA)

Cancer antigens refer to any substance produced by the body in response to a tumor. Various cancer antigens have been proposed as biomarkers for numerous types of cancer, such as CA 19-9, CA-125, and CA 15-3. CA 19-9 (also called carbohydrate antigen) refers to a specific antibody that binds a sialyl compound produced by cancer tissue (Sialyl Lewis A). CA 19-9 is elevated in several different types of cancer, such as adenocarcinomas or colorectal cancer (Magnani, 2004). CA-125 is a glycoprotein produced in fetal tissue as well as mesothelial cells in adults (Isaksson et al., 2017). Its function is thought to assist with cell adhesion, metastasis, and immunosuppression (Dorigo & Berek, 2011).

Kim et al. (2017) performed a study assessing the association of serum CA 19-9 and carcinoembryonic antigen (CEA) with colorectal neoplasia. A total of 124509 measurements of serum CEA level and 115833 measurements of serum CA 19-9 were taken. All subjects were asymptomatic and underwent a colonoscopy. Elevated serum levels of CEA were found to be associated with any adenoma. Elevated CA 19-9 was found to be associated with high-risk or advanced adenoma, CRC, and advanced colorectal neoplasia (Kim et al., 2017).

A study was performed by Feng et al. (2017) that focused on the diagnostic and prognostic value of CEA, CA 19-9, AFP, and CA-125 for early gastric cancer. The authors evaluated 587 patients and the positive rate for all markers combined was 10.4%. CEA's positive rate was 4.3%, CA 19-9's was 4.8%, AFP's was 1.5%, and CA-125's was 1.9%. The authors noted that elevated CEA was correlated with lymph node metastasis and concluded that CEA was an independent risk factor for poor prognosis of early gastric cancer (Feng et al., 2017).

Lucarelli et al. (2014) evaluated CA 15-3, CA-125, and B2M as biomarkers for renal cell carcinoma (RCC). A total of 332 patients undergoing nephrectomy for RCC were analyzed. The authors found that 35.2%

(117/332) of patients had abnormal levels of CA 15-3, 9.6% (32/332) had abnormal levels of CA-125, and 30.4% (101/332) had abnormal B2M. Cancer specific survival (CSS) rates significantly decreased for high levels of any of the three biomarkers, and at a multivariate analysis high levels of CA 15-3 were found to be an independent adverse prognostic risk factor for CSS (Lucarelli et al., 2014).

Chen et al. (2018) analyzed four serum tumor markers in patients with ovarian tumors. Human epididymis protein four (HE4), CA-125, CA19-9, and CEA were all studied. The authors evaluated 386 healthy controls, 262 patients with benign ovarian tumors, and 196 patients with malignant ovarian tumors. The authors found that the serum marker levels were significantly higher in patients with malignant tumors than the two other groups. HE4 was found to have a high specificity (96.56%) in malignant tumors. HE4, CA-125, CA19-9, and CEA had sensitivities of 63.78%, 62.75%, 35.71%, and 38.78%, respectively. HE4 and CA-125 combined were found to have the highest diagnostic sensitivity at 80.10%, as well as a specificity of 69.08%. Although adding markers to the HE4/CA-125 combination increased diagnostic sensitivity (to 88.52%), this difference was not considered significant (Chen et al., 2018).

Isaksson et al. (2017) performed a study of tumor markers' association with resectable lung adenocarcinomas. The study evaluated blood samples from 107 patients with stages I-III lung adenocarcinoma and examined the following markers: CEA, CA 19-9, CA-125, HE4, and neuron-specific enolase (NSE). When the authors calculated the disease-free survival rate, CA 19-9 and CA-125 were found to be significantly associated with recurrent disease with a combined hazard ratio of 2.8. The authors stated that "high pre-operative serum CA 19-9 and/or CA 125 might indicate an increased incidence of recurrent disease in resectable lung adenocarcinomas" (Isaksson et al., 2017).

Bind et al. (2021) evaluated the diagnostic performance of CA19-9 and CA-125 for gallbladder cancers. A total of 118 patients were included; 91 benign cases and 27 malignant. The mean value of CA19-9 was found to be 12.86 U/mL in benign cases and 625.35 U/mL in malignant cases. For CA-125, the mean value for benign cases was found to be 17.98 U/mL and for malignant cases, 239.63 U/mL. The authors examined a theoretical diagnostic cut-off value of 252.31 U/mL for CA19-9 and 92.19 U/mL for CA-125. At this cutoff, sensitivity and specificity for CA19-9 were 100% and 98.9% respectively, and for CA-125, 100% and 94.5%. The authors concluded that "…both serum CA 19-9 and serum CA 125 may act as a good adjunct for diagnosis of cases of carcinoma gallbladder along with imaging studies. However, changes in CA19-9 are more significant than CA 125" (Bind et al., 2021).

Carcinoembryonic antigen (CEA)

Carcinoembryonic antigen is a protein normally produced by fetal tissue, and as with AFP, stabilizes soon after birth. CEA is often elevated in malignancies such as breast or pancreatic cancer, although other conditions such as liver damage or cigarette smoking may affect CEA levels as well (Li, 2024). The gene encoding CEA encompasses certain genes encoding for cell adhesion, as well as MHC antigens (Duffy, 2001).

Chromogranin A (CgA)

Chromogranins are proteins contained in neurosecretory vesicles of NET cells and are typically elevated in neuroendocrine neoplasms. CgA is the most sensitive of the three chromogranins, and as such as the

primary marker used to evaluate neoplasms. However, this biomarker is highly variable (Strosberg, 2024).

A meta-analysis performed by Yang et al. (2015) assessed the association of CgA with neuroendocrine tumors. The analyses included 13 studies totaling 1260 patients (967 healthy controls), and the pooled sensitivity was found to be 0.73. The pooled specificity was found to be 0.95. However, the study stressed that further research needs to be undertaken (Yang et al., 2015). Another study by Tian et al. (2016) found that although median CgA levels were significantly higher than healthy controls (93.8 ng/mL compared to 37.1 ng/mL), only a weak correlation was found between changes in serum CgA levels and clinical regimen. The CgA cutoff value for this study was 46.2 ng/mL, which led to a sensitivity of 78.8% and specificity of 73.8% (Tian et al., 2016).

Inhibins

The primary function of inhibins is to inhibit hormones such as follicle stimulating hormone. However, since this protein is restricted to ovarian granulosa cells in individuals with ovaries, unusual levels of inhibins may signal tumors in this region (Walentowicz et al., 2014). This marker exists as two different isoforms: inhibin A and B. Either form can be measured, although an active tumor may over-secrete one or both forms (Gershenson, 2022). Inhibin B is generally considered to be more accurate than inhibin A, with sensitivities ranging from 0.88 to 1.00 whereas inhibin A's sensitivity ranges from 0.67-0.77. However, inhibin B has limitations of its own such as fluctuations with the menstrual cycle (Farkkila et al., 2015).

Farkkila et al. (2015) evaluated anti-Müllerian hormone (AMH) and inhibin B in the context of ovarian adult-type granulosa cell tumors (AGCTs). The study included 560 samples taken from 123 patients, and both markers were significantly elevated in AGCTs. The area under the curve for inhibin B was 0.94, but measurement of both markers was noted to be a better method than measuring either marker individually (Farkkila et al., 2015).

Lactate Dehydrogenase (LDH)

Lactate Dehydrogenase is an enzyme that catalyzes the interconversion between lactate and pyruvate. LDH is often found to be upregulated in tumors and a key feature of cancer sites is the accumulation of lactate or lactic acid. This is thought to be caused by increased glycolysis and the increase in lactate causes an elevated concentration of LDH (Pucino et al., 2017). Increased LDH is found in several different cancers, such as B-cell lymphomas and osteosarcomas (NCCN, 2024a).

Liu et al. (2016) performed a study evaluating the OS rates of an extremely high concentration of LDH (>1000 IU/L, considered by the study to be four times the upper normal limit). A total of 311 patients with >1000 U/L were examined, and the OS rate of this cohort was 1.7 months with 163 perishing within two months. However, 51 patients' LDH decreased to normal following chemotherapy and the OS rate of this group was 22.6 months. The cohort who survived at two months but did not see their LDH decrease had an OS rate of four months. There was no positive association found between OS and type of cancer, although there were different OS rates for patients at different stages of lymphoma (Liu et al., 2016).

Serum free light chains

Light chains are proteins produced by plasma cells that, along with heavy chains, collectively make up an immunoglobulin macromolecule. There are a total of five heavy chain protein classes (IgG, IgE, IgA, IgD, and IgM), and two light chain protein classes (kappa and lambda). Healthy plasma cells produce polyclonal immunoglobulins that are capable of binding to antigens and inducing an immune response; unhealthy plasma cells produce monoclonal immunoglobulins that do not effectively engage antigens (Kyrtsonis MC, 2012). In the case of certain plasma cell disorders, an abundance of monoclonal immunoglobulin or free light chains (kappa and/or lambda) may accumulate in the serum and serve as useful diagnostic markers.

For example, multiple myeloma is an uncontrolled growth of plasma cells (ACS, 2018a). In most cases, the cancerous clonal cells secrete an intact monoclonal immunoglobulin, where the gold standard for diagnosis is serum protein electrophoresis and immunofixation (Tosi et al., 2013). Less commonly, however, myeloma clones will secrete only light chains; in these instances, a serum free light chain assay can be employed to quantify the ratio of kappa and lambda chains in the serum. It has been demonstrated that in healthy individuals, the kappa/lambda ratio in the serum is approximately 0.58 (Katzmann et al., 2002). In the case of plasma cell neoplasms, free light chains are overproduced, and the kidneys are unable to completely clear them, resulting in accumulation in the serum and a change in the kappa/lambda ratio. This ratio is often used to aid in the diagnosis, prognosis, and monitoring of plasma cell disorders (Tosi et al., 2013).

Waldenström's Macroglobulinemia (WM) is a type of cancer that is similar to multiple myeloma and non-Hodgkin lymphoma. WM cells are called "lymphoplasmacytoid" because they have features of both plasma cells and lymphocytes (ACS, 2018b). WM cells are distinguished by the production of immunoglobulin M (IgM) serum monoclonal protein, also referred to as a "macroglobulin" (Cautha et al., 2022). While serum IgM level is useful for diagnostic purposes, it does not correlate with prognosis. The addition of a serum free light chain assay to the care of patients with suspected Waldenström's Macroglobulinemia has been postulated to improve overall care, as it may help differentiate patients with another, potentially benign disorder called monoclonal gammopathy of undetermined significance (MGUS), as well as influence prognosis (Moreau AS, 2006).

Castleman disease represents a group of B-cell lymphoproliferative disorders characterized by distinct pathogenesis and clinical outcomes (Oyaert et al., 2014; D. Wu et al., 2018). Patients with suspected Castleman disease have been reported to present with abnormal levels or kappa or lambda light chains, making the serum free light chain assay a potentially useful tool in the management of this disease (Oyaert et al., 2014; D. Wu et al., 2018). Utilization of a serum free light chain assay has been reported to be clinically useful in the workup of Castleman disease, though an important caveat is that changes in the absolute values of both kappa and lambda free light chain in the serum can occur with preservation of a ratio within the normal reference range (Stankowski-Drengler et al., 2010); hence, both the free light chain ratio as well as the absolute values of each light chain protein should be considered.

Immunoglobulin light chain amyloidosis is a disorder that results from the accumulation of amyloid fibrils due to the production of fragments of monoclonal light chains (Dispenzieri, 2024; Merlini et al., 2013). As amyloid fibrils continue to accumulate, they begin to interfere with the biological function of various organs, eventually resulting in organ damage and potentially organ failure. Due to the involvement of light chains in the pathogenesis of amyloidosis, serum free light chain measurement may

hold diagnostic and prognostic value, and be a viable response marker following therapy (Akar et al., 2005; Bhole et al., 2014; Kumar et al., 2010).

Importantly, Bhole et al. (2014) highlighted key challenges with serum free light chain assays that include but are not limited to over or under-estimation of the monoclonal protein, and performance differences between available tests. Therefore, despite the demonstrated utility of these assays, clinicians should be aware of their limitations.

Troponin

Troponins are proteins that reside in muscle cells and function as part of the protein complex responsible for generating muscular contraction and relaxation (Chaulin, 2022). Two forms of troponin (troponin I [TnI] and troponin T [TnT]) have particular utility as biomarkers of cardiac dysfunction or damage due to their relative abundance in cardiac cells (Sharma et al., 2004). Accordingly, TnI and TnT have been studied as potentially useful markers for the management of various disorders that affect the heart, including systemic light chain amyloidosis. Persistently elevated cardiac troponin levels are frequently observed in individuals with amyloidosis and can serve as an indicator of cardiac amyloid infiltration (Perfetto et al., 2014).

Tryptase

Tryptases are tetrameric enzymes and one of the major types of protease found in mast cells, which play an integral role in the allergic and inflammatory responses (Payne & Kam, 2004; Pejler et al., 2010). Normal allergic responses involve the release of these proteases in addition to other active mediators including histamine, serotonin, lysosomal enzymes, and proteoglycans (Leru, 2022), which can be measured in an individual's tissue or serum. These mediators can thus serve as useful markers for disorders involving mast cell production and activation, such as systemic mastocytosis, where serum tryptase is an accepted diagnostic criterion (AAAAI).

Urokinase plasminogen activator (uPA)

Urokinase plasminogen activator is a serine protease with an important role in cancer invasion and metastasis (Stephens et al., 1998). When bound to its receptor (uPAR), uPA converts plasminogen into plasmin and mediates degradation of the extracellular matrix during tumor cell invasion. High levels have been associated with shorter survival in individuals with breast cancer (Chappuis et al., 2001; Foekens et al., 2000; Malmstrom et al., 2001; Stephens et al., 1998). American Society of Clinical Oncology guidelines include recommendations for the appropriate clinical situations in which measurement of uPA may be helpful (Foukakis & Bergh, 2022; Harris et al., 2016).

Proteomics

Proteomics is a qualitative and quantitative assessment of the protein constituents in a biological sample. This is typically performed with modification of polyacrylamide gel electrophoresis (PAGE) or matrix-assisted laser desorption/ionization (MALDI). However, this method is still under investigation (Raby, 2023).

Proteomic analyses have been performed in cancer patients to assess unusual levels of protein regulation. A study by Chen et al. (2017) evaluated the proteomes of patients with CRC and healthy controls. The investigators found thirty-six proteins that were upregulated in cancer patients as well as twenty-two proteins that were downregulated compared to healthy controls. The proteins that were upregulated tended to be involved in processes that regulated the "pretumorigenic microenvironment for metastasis" and the downregulated proteins tended to be ones that controlled tumor growth and cell survival (Chen et al., 2017).

Qin et al. (2020) performed a "serological proteome analysis" to explore the association between an identified protein marker and gastric cancer (GC). Proteomic analysis was used to identify the protein marker of interest, an autoantibody called "anti-GRP78" (along with its corresponding antigen, the 78-kDa glucose-regulated protein [GRP78]). Two cohorts were included, a test group of 266 patients (133 GC patients, 133 controls) and a validation group of 600 patients (300 GC, 300 control). The authors found that the level of anti-GRP78 was higher in both cohorts. The receiver operating characteristic (ROC) curve analysis found similar values for both groups to identify GC patients among control patients. The AUC ranged from 0.676 to 0.773 in the test group and 0.645 to 0.707 in the validation group. The authors noted this marker's potential diagnostic use (Qin et al., 2020).

Guidelines and Recommendations

National Comprehensive Cancer Network (NCCN)

The NCCN provides a Biomarkers Compendium to "support decision-making around the use of biomarker testing in patients with cancer" (NCCN, 2023), which serves as a primary source of guidance for coverage criteria in this policy. The Biomarkers Compendium may be accessed through nccn.org.

In the most recently published clinical practice guidelines for ovarian cancer, NCCN states they recommend "that all patients with suspected ovarian malignancies (especially those with an adnexal mass) should undergo evaluation by an experienced gynecologic oncologist prior to surgery" (NCCN, 2024d). "A number of specific biomarkers and algorithms using multiple biomarker test results have been proposed for preoperatively distinguishing benign from malignant tumors in patients who have an undiagnosed adnexal/pelvic mass. Biomarker tests developed and evaluated in prospective trials comparing preoperative serum levels to postoperative final diagnosis include serum HE4 and CA-125, either alone or combined using the Risk of Ovarian Malignancy Algorithm [ROMA] algorithm; the MIA (brand name OVA1) based on serum levels of five markers: transthyretin, apolipoprotein A1, transferrin, beta-2 microglobulin, and CA-125; and the second-generation MIA (MIA2G, branded name OVERA) based on CA-125, transferrin, apolipoprotein A1, follicle-stimulating hormone [FSH], and HE4. The FDA has approved the use of ROMA, OVA1, or OVERA for estimating the risk for ovarian cancer in those with an adnexal mass for which surgery is planned, and have not yet been referred to an oncologist. Although the American Congress of Obstetricians and Gynecologists (ACOG) has suggested that ROMA and OVA1 may be useful for deciding which patients to refer to a gynecologic oncologist, other professional organizations have been non-committal. Not all studies have found that multi-biomarker assays improve all metrics (ie, sensitivity, specificity, positive predictive value, negative predictive value) for prediction of malignancy compared with other methods (eg, imaging, single-biomarker tests, symptom index/clinical assessment). Currently, the NCCN Panel does not recommend the use of these biomarker tests for determining the status of an undiagnosed adnexal/pelvic mass" (NCCN, 2024d).

American Society of Clinical Oncology (ASCO)

Clinical Practice Guideline on Uses of Serum Tumor Markers (STMs) in Adult Males with Germ Cell Tumors (GCTs) were released in 2010 (Gilligan et al., 2010). ASCO recommends against any STMs to screen for GCTs. While ASCO recommends assessment of serum AFP and hCG before orchiectomy to establish a diagnosis and baseline levels, it recommends against its use to decide whether to perform an orchiectomy. The society also recommends against using these biomarkers to "guide treatment of patients with CUP and indeterminate histology." However, substantially elevated serum AFP and/or hCG may be considered sufficient for a diagnosis in unusual cases such as patients presenting with a retroperitoneal or anterior mediastinal primary tumor. Their recommendations also include measuring serum AFP, hCG, and LDH for "all patients with testicular nonseminomatous germ cell tumors (NSGCTs) shortly after orchiectomy and before any subsequent treatment", "before chemotherapy begins for those with mediastinal or retroperitoneal NSGCTs to stratify risk and select treatment", and "immediately prior to chemotherapy for stage II/III testicular NSGC" (Gilligan et al., 2010).

The society recommends measuring AFP and hCG before retroperitoneal lymph node dissection in patients with stage I or II NSGCT and recommends measuring serum AFP and hCG at the start of each chemotherapy cycle and when chemotherapy concludes. These biomarkers are also recommended to be measured during surveillance after "definitive therapy for NGSCT" and this surveillance should continue for 10 years after therapy concludes (Gilligan et al., 2010).

Measuring "postorchiectomy serum concentrations of hCG and/or LDH for patients with testicular pure seminoma and preorchiectomy elevations" was also discussed, but ASCO recommends against using these concentrations for staging or prognosis. No markers are recommended to guide treatment decisions, monitor response, or progression for seminomas. However, serum hCG and AFP should be measured both when treatment concludes as well as during post-treatment surveillance. ASCO recommends these intervals: every two to four months in the first year, every three to four months in the second year, every four to six months in the third and fourth years, and annually thereafter. Surveillance should last for at least 10 years following the conclusion of therapy (Gilligan et al., 2010).

Guidelines were released on the use of biomarkers to inform treatment decisions regarding systemic therapy for women with metastatic breast cancer. "Patients with accessible, newly diagnosed metastases from primary breast cancer should be offered biopsy for confirmation of disease process and testing of ER, PR, and HER2 status. With discordance of results between primary and metastatic tissues, the panel consensus is to preferentially use the ER, PR, and HER2 status from the metastasis to direct therapy if supported by the clinical scenario and the patient's goals for care." Decisions on changing to a new drug or regimen, initiating, or discontinuing treatment should be based on the patient's goals for care and clinical evaluation and judgment of disease progression or response. There is no evidence at this time that changing therapy solely based on tissue or circulating biomarker results beyond ER, PR, and HER2 improves health outcomes, quality of life, or cost-effectiveness. To date, clinical utility has not been demonstrated for any additional biomarkers. "CEA, CA 15-3, and CA 27.29 may be used as adjunctive assessments to contribute to decisions regarding therapy for metastatic breast cancer. Data are insufficient to recommend use of CEA, CA 15-3, and CA 27.29 alone for monitoring response to treatment" (Van Poznak et al., 2015).

A provisional clinical opinion on evaluating susceptibility to pancreatic cancer was released by ASCO, stating that "there are currently no proven biomarkers using noninvasively obtained biospecimens (eg, blood, urine, stool) for early detection of pancreatic cancer in asymptomatic individuals." ASCO states that further validation of biomarkers is needed (Stoffel et al., 2018).

Finally, a guideline on treatment of malignant pleural mesothelioma was published, stating that calretinin, keratins five and six, and nuclear WT-1 are expected to be positive while CEA, EPCAM, Claudin four, and TTF-1 should be negative. Non-tissue based biomarkers are currently not recommended due to their unvalidated statistical accuracy (Kindler et al., 2018).

Association for Diagnostics & Laboratory Medicine (ADLM); formerly the National Academy of Clinical Biochemistry (NACB) and AACC Academy

Practice guidelines on the use of tumor markers for liver, bladder, cervical, and gastric cancers were released by ADLM (Sturgeon et al., 2010). The association recommends use of AFP measurements when managing hepatocellular carcinoma (HCC). For screening, ADLM recommends AFP be measured at 6-month intervals in patients at high risk of HCC, noting that concentrations above 20 µg/L should "prompt further investigation even if an ultrasound is negative." Sustained increases of serum AFP may be used in combination with ultrasound to inform detection and management and AFP concentrations may provide prognostic information in untreated patients. Monitoring of disease should include measurement of AFP. However, other liver biomarkers such as Glypican-3 cannot be recommended at this time without further research (Sturgeon et al., 2010).

The association did not recommend any biomarkers for the management of bladder cancer (such as NMP22, UroVysion, etc.), stating that further research is required to assess their utility. ADLM did not recommend any biomarkers for screening, monitoring, prognosis, or diagnosis of cervical cancer. While pretreatment measurements of squamous cell carcinoma antigen (SCC) were acknowledged to provide information, their routine use could not be recommended. ADLM did not recommend any biomarkers for screening, diagnosis, or prognosis of gastric cancer. Routine measurement of CEA or CA 19-9 was also not recommended (Sturgeon et al., 2010).

Guidelines on use of tumor markers for testicular, prostate, colorectal, breast, and ovarian cancers were also released by ADLM (C. M. Sturgeon et al., 2008). For testicular cancer, ADLM stated that pretreatment determination of AFP, lactate dehydrogenase (LDH), and human chorionic gonadotropin (hCG) was mandatory if testicular cancer was suspected or if risk stratification and staging was done. These three biomarkers were also recommended for monitoring. ADLM notes that measurement of the free β-subunit of human chorionic gonadotropin (hCGβ) component is essential when measuring hCG. For prostate cancer, PSA assessment is required during all stages of the disease, with ADLM recommending against age-specific intervals. PSA measuring is recommended to monitor disease status after treatment. However, ADLM did not make any recommendations on PSA screening for prostate cancer (C. M. Sturgeon et al., 2008).

For colorectal cancer (CRC), carcinoembryonic antigen (CEA) measurement is recommended every 3 months in stage II or III if "patient is a candidate for surgery or systemic therapy of metastatic disease." Pre-operative CEA measurements may be used in conjunction with other factors to plan surgery. Regular CEA measurements should be done in patients with advanced CRC that are undergoing systemic

therapy. However, CEA is not recommended for screening in healthy individuals. Routine measurement of other biomarkers such as CA 19-9, TIMP-1, or CA 242 is not recommended for prognosis or predicting response to treatment. ADLM recommends individuals older than 50 be screened for CRC. Fecal DNA is also recommended for CRC screening, as joint guidelines from other societies such as the American Cancer Society have recommended its use. Finally, ADLM supports guidelines such as the NCCN and AGA regarding genetic testing for CRC (C. M. Sturgeon et al., 2008).

According to ADLM, estrogen receptor (ER) and progesterone receptor (PR) measurements should be done in all patients diagnosed with breast cancer. HER-2 should be measured in all patients with invasive breast cancer, while urokinase plasminogen activator (uPA) and plasminogen activator inhibitor 1 (PAI-1) may be used to identify "lymph node–negative breast cancer patients who do not need or are unlikely to benefit from adjuvant chemotherapy." CA 15-3, CEA, and BR 27.29 should not routinely be used for early detection in asymptomatic patients with diagnosed breast cancer. *BRCA1* and *BRCA2* mutation testing may be used to identify women at high risk of developing breast or ovarian cancer, while OncoType DX may be used to predict recurrence in "lymph node–negative, ER-positive patients receiving adjuvant tamoxifen." ADLM does recommend that microarray-based gene signatures should be routinely used for predicting patient outcome (C. M. Sturgeon et al., 2008).

For ovarian cancer, CA-125 screening is not recommended for asymptomatic women but is recommended (with transvaginal ultrasound) for early detection of ovarian cancer in women with hereditary syndromes. CA-125 is also recommended for distinguishing benign from malignant masses and may be used to monitor chemotherapeutic response. Measurement of CA-125 during follow-up visits is recommended if the patient's initial values were increased. CA-125 measurement is also recommended during primary therapy. Other biomarkers such as inhibin and hCG cannot be recommended at this time (C. M. Sturgeon et al., 2008).

Addressing serum free light chains, ADLM recommends ordering serum free light chain testing (with serum protein electrophoresis and immunofixation) when screening for patients suspected of having a malignant monoclonal process: multiple myeloma (MM), Waldenstrom macroglobulinemia, B-cell lymphoproliferative process, AL amyloidosis, or monoclonal gammopathy of renal significance (MGRS). When it comes to prognosis, the ADLM recommends using serum light chains as a baseline measurement to assess the risk of all plasma cell disorders. For monitoring, the ADLM recommends using serum light chains to determine complete stringent remission; to follow patients with oligosecretory multiple myeloma and an abnormal serum free light chain ratio; and to follow AL amyloidosis with an abnormal serum free light chain ratio (ADLM, 2024).

North American Neuroendocrine Tumor Society (NANETS)

The North American Neuroendocrine Tumor Society notes that although most of its expert panel's members measure CgA and/or pancreastatin, a majority of them believed that "these tumor markers assist in patient management only occasionally or rarely." No consensus was reached on whether these tumor markers should be routinely measured (NANETS, 2017).

In 2020, NANETS published a guideline focusing on the "Surveillance and Medical Management of Pancreatic Neuroendocrine Tumors." In it, they authors remark that "Use of nonspecific tumor markers such as CgA, pancreastatin (PcSt), and others is not recommended for routine use in patients with

pNETs," stating that these marker analyses "rarely, if ever" influence treatment (Halfdanarson et al., 2020).

American Association for the Study of Liver Diseases (AASLD)

The American Association for the Study of Liver Diseases provided updated guidance on the prevention, diagnosis, and treatment of hepatocellular carcinoma in May 2023. This guideline states that several promising biomarkers are being investigated for potential utility in HCC surveillance, but most have not been sufficiently validated for this purpose, with the exception of AFP-L3% and DCP. Hence, "AASLD does not recommend routine use of CT- or MRI-based imaging and tumor biomarkers, outside of AFP, for HCC surveillance in at-risk patients with cirrhosis or chronic HBV (Level 5, Weak Recommendation)." While AFP may be used for screening purposes, AASLD does not yet support its diagnostic use, stating that "the diagnosis of HCC should be based on noninvasive imaging criteria or pathology. Biomarkers, such as AFP, are not sufficiently accurate to make a diagnosis of HCC" (Singal et al., 2023). Finally, AASLD advises use of the BCLC (Barcelona Liver Clinic Cancer) system for disease staging, which incorporates AFP levels.

The association also published updated guidance on primary sclerosing cholangitis and cholangiocarcinoma in February 2023. This guideline acknowledges that CA 19-9 is the most common serum marker associated with cholangiocarcinoma (CCA), but is limited by variable sensitivity and specificity, particularly because it may be elevated in many benign and other malignant conditions (Bowlus et al., 2023).

American Thyroid Association (ATA)

The American Thyroid Association cannot recommend for or against routine measurement of serum calcitonin in patients with thyroid nodules. Furthermore, ATA cautions that unusual levels of calcitonin may occur with a variety of other conditions apart from medullary thyroid carcinoma, and notes that calcitonin levels are often elevated in young children and males compared to females (Haugen et al., 2016; Wells et al., 2015).

Regarding management of patients following thyroidectomy for persistent or recurrent medullary thyroid carcinomas, measurement of serum calcitonin does play an important role. Along with a physical exam, serum calcitonin levels, CEA, TFTs, and TSH should be measured every 6 to 12 months. Depending on these biomarker levels, further action may be warranted (ATA, 2017).

International Mesothelioma Interest Group

The Interest Group considers the following biomarkers to be "very useful": Calretinin Cytokeratin 5/6, WT1, Podoplanin (D2-40) (for epitheloid mesothelioma), Claudin four, MOC31, B72.3, CEA, BER-EP4, BG8 ($Lewis^Y$), TTF-1, and Napsin A (for lung adenocarcinoma) (Husain et al., 2018).

European Society for Medical Oncology (ESMO): Malignant pleural mesothelioma

For epithelioid mesotheliomas, "diagnosis can usually be made by using a combination of two 'mesothelioma-associated' markers [e.g. calretinin, Wilms' tumour-1 (WT-1), cytokeratin 5/6] and two

'(adeno)carcinoma-associated' markers [e.g. CEA, Ber-EP4, MOC-31], supplemented by other markers dependent on possibility of known, suspected or occult malignancies" (Popat et al., 2022).

Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, please visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

There are numerous FDA-approved tests for the assessment of serum tumor markers. Additionally, many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid Services (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
81479	Unlisted molecular pathology procedure
81500	Oncology (ovarian), biochemical assays of two proteins (CA-125 and HE4), utilizing serum, with menopausal status, algorithm reported as a risk score Proprietary test: Risk of Ovarian Malignancy Algorithm (ROMA)™ Lab/manufacturer: Fujirebio Diagnostics
81503	Oncology (ovarian), biochemical assays of five proteins (CA-125, apolipoprotein A1, beta-2 microglobulin, transferrin, and pre-albumin), utilizing serum, algorithm reported as a risk score Proprietary test: OVA1™ Lab/manufacturer: Vermillion, Inc
81538	Oncology (lung), mass spectrometric 8-protein signature, including amyloid A, utilizing serum, prognostic and predictive algorithm reported as good versus poor overall survival Proprietary test: VeriStrat® Lab/manufacturer: Biodesix, Inc
81599	Unlisted multianalyte assay with algorithmic analysis
82105	Alpha-fetoprotein (AFP); serum
82107	Alpha-fetoprotein (AFP); AFP-L3 fraction isoform and total AFP (including ratio)
82232	Beta-2 microglobulin
82308	Calcitonin
82378	Carcinoembryonic antigen (CEA)
83520	Immunoassay for analyte other than infectious agent antibody or infectious agent antigen; quantitative, not otherwise specified
83521	Immunoglobulin light chains (ie, kappa, lambda), free, each
83615	Lactate dehydrogenase (LD), (LDH);

CPT	Code Description
83789	Mass spectrometry and tandem mass spectrometry (eg, MS, MS/MS, MALDI, MS-TOF, QTOF), non-drug analyte(s) not elsewhere specified, qualitative or quantitative, each specimen
83880	Natriuretic peptide
83950	Oncoprotein; HER-2/neu
83951	Oncoprotein; des-gamma-carboxy-prothrombin (DCP)
84075	Phosphatase, alkaline
84078	Phosphatase, alkaline; heat stable (total not included)
84080	Phosphatase, alkaline; isoenzymes
84484	Troponin, quantitative
84702	Gonadotropin, chorionic (hCG); quantitative
84703	Gonadotropin, chorionic (hCG); qualitative
84704	Gonadotropin, chorionic (hCG); free beta chain
84999	Unlisted chemistry procedure
86300	Immunoassay for tumor antigen, quantitative; CA 15-3 (27.29)
86301	Immunoassay for tumor antigen, quantitative; CA 19-9
86304	Immunoassay for tumor antigen, quantitative; CA 125
86305	Human epididymis protein 4 (HE4)
86316	Immunoassay for tumor antigen, other antigen, quantitative (eg, CA 50, 72-4, 549), each
86336	Inhibin A
0003U	Oncology (ovarian) biochemical assays of five proteins (apolipoprotein A-1, CA 125 II, follicle stimulating hormone, human epididymis protein 4, transferrin), utilizing serum, algorithm reported as a likelihood score Proprietary test: Overa™ (OVA1 Next Generation) Lab/manufacturer: Aspira Labs, Inc, Vermillion, Inc
0092U	Oncology (lung), three protein biomarkers, immunoassay using magnetic nanosensor technology, CPTsma, algorithm reported as risk score for likelihood of malignancy Proprietary test: REVEAL Lung Nodule Characterization Lab/Manufacturer: MagArray, Inc
0163U	Oncology (colorectal) screening, biochemical enzyme-linked immunosorbent assay (ELISA) of 3 plasma or serum proteins (teratocarcinoma derived growth factor-1 [TDGF-1, Cripto-1], carcinoembryonic antigen [CEA], extracellular matrix protein [ECM]), with demographic data (age, gender, CRC-screening compliance) using a proprietary algorithm and reported as likelihood of CRC or advanced adenomas Proprietary test: BeScreened™-CRC Lab/Manufacturer: Beacon Biomedical Inc
0404U	Oncology (breast), semiquantitative measurement of thymidine kinase activity by immunoassay, serum, results reported as risk of disease progression Proprietary test: Divitum® Tka Lab/Manufacturer: Biovica Inc
G0327	Colorectal cancer screening; blood-based biomarker

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

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Revision History

Revision Date	Summary of Changes
09/04/2024	Reviewed and Updated: Updated the background, guidelines and recommendations, and evidence-based scientific references. Based on guidance from the National Comprehensive Cancer Network's Biomarker's Compendium, the following coverage additions and removals were made: **Alpha fetoprotein:** For "Ovarian cancers (less common)", added indication for Carcinosarcoma (malignant mixed mullerian tumors) to include monitoring/follow-up; clear cell carcinoma of the ovary to include monitoring/follow-up; grade 1 endometrioid carcinoma: monitoring/follow-up; mucinous neoplasms of the ovary to include monitoring/follow-up; low-grade serous carcinoma to include monitoring/follow-up. Removed the "(less common)" designation from Ovarian cancer row that has "Borderline epithelial tumors" following it. **Beta-2 microglobulin (B2M):** For chronic lymphocytic leukemia/small lymphocytic lymphoma, added indications for prognostic and/or therapy determination.

	Calcitonin (CALCA): For adenocarcinoma, and anaplastic/undifferentiated epithelial tumors added indication of workup. For occult primary (unknown primary cancer) added indication for workup. **Cancer antigen 15-3 and 27.29 (CA 15-3 and 27.29):** for occult primary cancers (cancers of unknown primary origin) added indications for assessing disease prognosis; and monitoring/follow-up for response. **Cancer antigen 19-9 (CA 19-9):** for occult primary cancers, added indications for assessing disease prognosis and monitoring/follow-up for response. For "Ovarian cancers (less common)", added indication for Carcinosarcoma (malignant mixed mullerian tumors) to include monitoring/follow-up; clear cell carcinoma of the ovary to include monitoring/follow-up; grade 1 endometrioid carcinoma: monitoring/follow-up; mucinous neoplasms of the ovary to include monitoring/follow-up; low-grade serous carcinoma to include monitoring/follow-up. Removed the "(less common)" designation from Ovarian cancer row that has "Borderline epithelial tumors" following it. For small bowel adenocarcinoma, added to other indications "at metastasis or recurrence." **Cancer antigen 125 (CA-125):** For "Ovarian cancers (less common)", added indication for Carcinosarcoma (malignant mixed mullerian tumors) to include monitoring/follow-up; clear cell carcinoma of the ovary to include monitoring/follow-up; grade 1 endometrioid carcinoma: monitoring/follow-up; mucinous neoplasms of the ovary to include monitoring/follow-up; low-grade serous carcinoma to include monitoring/follow-up. Removed the "(less common)" designation from Ovarian cancer row that has "Borderline epithelial tumors" following it. For uterine neoplasms added indication for "initial workup." **Carcinoembryonic antigen (CEA):** For gallbladder cancer added indication "of adjuvant treatment (as clinically indicated)" For "Ovarian cancers (less common)", added indication for Carcinosarcoma (malignant mixed mullerian tumors) to include monitoring/follow-up; clear cell carcinoma of the ovary to include monitoring/follow-up; grade 1 endometrioid carcinoma: monitoring/follow-up; mucinous neoplasms of the ovary to include monitoring/follow-up; low-grade serous carcinoma to include monitoring/follow-up. Removed the "(less common)" designation from Ovarian cancer row that has "Borderline epithelial tumors" following it. **Inhibin (INHA):** For adrenocortical carcinoma added indication for workup. For "Ovarian cancers (less common)", added indication for carcinosarcoma (malignant mixed mullerian tumors) to include monitoring/follow-up; clear cell carcinoma of the ovary to include monitoring/follow-up; grade 1 endometrioid carcinoma: monitoring/follow-up; mucinous neoplasms of the ovary to include monitoring/follow-up; low-grade serous carcinoma to include monitoring/follow-up. Removed the "(less common)" designation from Ovarian cancer row that has "Borderline epithelial tumors" following it. **Lactate dehydrogenase (LDH):** For "Ovarian cancers (less common)", added indication for carcinosarcoma (malignant mixed mullerian tumors) to include monitoring/follow-up; clear cell carcinoma of the ovary to include monitoring/follow-up; grade 1 endometrioid carcinoma: monitoring/follow-up; mucinous neoplasms of the ovary to include monitoring/follow-up; low-grade serous carcinoma to include monitoring/follow-up. Removed the "(less common)" designation from Ovarian cancer row that has "Borderline epithelial tumors" following it. For systemic mastocytosis, added indications for initial diagnostic workup.

Testing for Alpha-1 Antitrypsin Deficiency

Policy Number: AHS – M2068 – Testing for Alpha-1 Antitrypsin Deficiency	Prior Policy Name and Number, as applicable: • M2068 Genetic Testing for Alpha-1 Antitrypsin Deficiency
Initial Presentation Date: 06/16/2015 Effective Date: February 1, 2025	

Policy Description

Alpha 1-antitrypsin deficiency (AATD) is a genetic disease that causes deficient or defective production of the alpha-1 antitrypsin (AAT) protease inhibitor that can affect the lungs, liver, and skin (Stoller, 2024). AAT deficiency results in unbalanced rapid breakdown of proteins, especially in the supporting elastic tissue of the lungs (NORD, 2024a).

Related Policies

Policy Number	Policy Title
	Not applicable

Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) For individuals who are suspected of having alpha-1 antitrypsin (AAT) deficiency, serum quantification of alpha-1 antitrypsin (AAT) protein **and** AAT phenotyping **or** AAT proteotyping (see Note 1) **MEETS COVERAGE CRITERIA** once per lifetime in **any** of the following situations:
   a) For symptomatic individuals 18 years or older with emphysema, COPD, or asthma.
   b) For individuals with unexplained liver disease (e.g., chronic hepatitis with or without cirrhosis, chronically elevated aminotransferase levels, portal hypertension, primary liver cancer).
   c) For individuals with persistent obstruction on pulmonary function tests without identifiable risk factors (e.g., cigarette smoking, occupational exposure).
   d) For individuals 18 years or older with necrotizing panniculitis.
   e) For the siblings of an individual with known alpha-1 antitrypsin (AAT) deficiency.
   f) For individuals with anti-proteinase three-positive vasculitis (C-ANCA [anti-neutrophil cytoplasmic antibody]-positive vasculitis).
   g) For individuals with bronchiectasis without evident etiology.
   h) For individuals with neonatal cholestasis.

2) For individuals who have negative genotype results for common variants or who have discordant results between AAT serum levels and proteotype, but for whom a clinical suspicion of AAT deficiency remains, isoelectric focusing/phenotyping **MEETS COVERAGE CRITERIA**.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

3) For all other situations not described above, testing for AAT deficiency **DOES NOT MEET COVERAGE CRITERIA**.

NOTES:

Note 1: AAT phenotyping should be performed using isoelectric focusing. AAT proteotyping (Pi-typing or protease inhibitor typing) for Z and S alleles should be performed using liquid chromatography-tandem mass spectrometry.

Table of Terminology

Term	Definition
A1AT	Alpha-1 antitrypsin
AAT	Aspartate aminotransferase
AATD	Alpha 1-antitrypsin deficiency
ACG	American College of Gastroenterology
ALT	Alanine transaminase
AST	Aspartate aminotransferase
ATS/ERS	American Thoracic Society/European Respiratory Society
C-ANCA	C-Anti-neutrophil cytoplasmic antibody
CMS	Centers for Medicare and Medicaid Services
COPD	Chronic obstructive pulmonary disorder

CTS	Canadian Thoracic Society
ERS	European Respiratory Society
GC	Gas chromatography
GOLD	Global Initiative for Chronic Obstructive Lung Disease
HPLC	High-performance liquid chromatography
LBV	Likely benign variants
LDTs	Laboratory developed tests
MALDI	Matrix-assisted laser desorption ionisation
MMP-12	*Matrix metalloproteinase-12* gene
MS-TOF	Time of flight mass spectrometry
NAFLD	Non-alcoholic fatty liver disease
NICE	National Institute Health and Care Excellence
NORD	National Organization for Rare Disorders
ON-CD	Ontario Center
PCR	Polymerase chain reaction
Pi	Protease inhibitor
PiMZ	Protease inhibitor Z allele
PV	Pathogenic variant
QTOF	Quadrupole time of flight
RCTs	Randomized controlled trials
RFLP	Restriction fragment length polymorphism
SERPINA1	Serine protease inhibitor
UMV	Undefined molecular variants
WHO	World Health Organization

Scientific Background

Alpha-1 antitrypsin deficiency (AATD) is an underrecognized genetic condition that affects approximately 1 in 2,000 to 1 in 5,000 individuals and predisposes to liver disease and early-onset emphysema (Stoller & Aboussouan, 2012). It is estimated (Campos et al., 2005) that up to 80,000 to 100,000 people in the United States have the severe form of the disease (homozygous in null or abnormal alleles). There is much variation in the disease prevalence in other nations (de Serres et al., 2007), but most current estimates are that three million people worldwide have severe AATD (Stoller, 2023).

Alpha-1 antitrypsin deficiency is a result of abnormal alpha-1 antitrypsin (AAT) protein inherited in an autosomal recessive pattern with codominant expression in which both genes inherited can be active and contribute to the genetic trait they control. AAT is a member of the serine protease inhibitor (Pi) family, referred to as "serpins", and it inhibits the proteolytic enzymes elastase, trypsin, chymotrypsin, and thrombin. AAT is encoded by the gene *SERPINA1* (Stoller, 2023).

The AAT protein is produced in the liver and has a role in protecting lungs from injury by neutrophil elastase, which is secreted by white blood cells as a response to inflammation or infection. If the enzyme remains unchecked by AAT protein, damage to alveoli resulting in chronic obstructive pulmonary

disease can occur. This includes emphysema, asthma, bronchiectasis, and spontaneous pneumothorax. Smoking and other environmental exposure can cause further damage (Stoller, 2023, 2024).

Abnormal molecules of AAT protein caused by this illness can also cause liver dysfunction. Pathologic polymerization of the variant AAT can occur, resulting in intrahepatocyte accumulation of AAT molecules, leading to cirrhosis, fibrosis, cholestasis, or hepatomegaly. Liver disease is more common in individuals with certain allele combinations. Gender and obesity may be risk factors for progression to advanced liver disease in adulthood among patients with severe AAT deficiency. In contrast, alcohol use and viral hepatitis do not appear to increase the risk of progressive hepatic failure (Stoller, 2024). AATD is a common genetic cause of liver disease in children (de Serres et al., 2003).

Skin manifestations of AATD are also recognized. The most associated skin condition is necrotizing panniculitis. In this condition, inflammatory skin lesions are thought to be a consequence of the AAT protein loss of function and subsequent unchecked proteolysis enzyme activity in the skin and subcutaneous tissue. Associations between alpha-1 antitrypsin (AAT) and vascular disease, inflammatory bowel disease, glomerulonephritis, and vasculitis have been proposed but not definitively established (Stoller, 2024).

Due to the numerous alleles associated with AAT, each allele has been given a letter code based on the "electrophoretic mobility of the protein produced". The normal allele is the "M" allele, and the most common mutation is the "Z" allele. This system applies for each individual allele; for example, a homozygous Z genotype would be denoted as "ZZ". Similarly, a wildtype (or "normal") genotype would be "MM". Besides the normal phenotype, the three other categories of AAT include "deficient" in which insufficient AAT is produced; "null" in which no AAT is produced at all; and "dysfunctional" in which a typical amount of AAT is produced, but the AAT protein does not function correctly (Stoller, 2023).

Laboratory testing for AATD is comprised of three strategies: serum or plasma AAT quantification, AAT protein phenotyping, and genotyping. Guidelines from national and international societies (e.g., World Health Organization, Spanish Society of Pneumology and Thoracic Surgery, European Respiratory Society) recommend testing for AATD at least once per lifetime for all individuals with liver disease of unknown etiology and for all individuals with chronic obstructive pulmonary disorder (COPD), emphysema, or adults with asthma and irreversible airflow obstruction (Belmonte et al., 2020).

Proprietary Testing

Initial testing often begins with serum quantification of AAT protein. This can be done through several methods, including immune turbidimetry and nephelometry (Stoller, 2023). A low level is generally represented by a serum level below 11 micromol/L (less than 57 mg/dl using nephelometry). Due to the variation of reference ranges in different testing methodologies, most labs will complete isoelectric phenotyping on any individual with a serum AAT levels of < 100 mg/dL (18.4 micromol/L). In fact, the American Thoracic Society suggests persons with borderline serum levels (defined as 12-35 micromoles or 90 to 140 mg/dL) have qualitative testing (ATS/ERS, 2003).

Isoelectric immunophenotype testing uses the difference in migration rates of allele variants under isoelectric focusing. For example, the M variant will migrate to the middle of the gel, Z will migrate the slowest, and F migrates quickly to the side closest to the anode. This is not a genetic test. On occasion the results can be inconclusive or discordant with quantitative testing, requiring genotype testing of the most-common variants (Stoller, 2023).

Genotype testing for the most common allele variants can be utilized where isoelectric immunophenotype testing is inconclusive. Usually polymerase chain reaction (PCR) or restriction fragment length polymorphism (RFLP) techniques are utilized to determine if the most common alleles are present. When dealing with the possibility of a rare variant or null allele, full gene sequencing can be utilized as a final diagnostic measure (Stoller, 2023).

In 2017, Grifols won FDA approval for AAT Deficiency Test, which is capable of simultaneously analyzing 99% of the most prevalent known mutations causing alpha-1 antitrypsin deficiency. The molecular test analyzes simultaneously 192 samples per kit, and in a single reaction, identifies 14 of the most prevalent known mutations in the SERPINA1 gene, responsible for this genetic disorder (Grifols, 2017).

Using Progenika's FDA-cleared A1AT Genotyping Test, Matrix Clinical Labs released the proprietary Alpha ID screening test, a comprehensive targeted genetic test assessing 14 common and rare alleles in the *SERPINA1* gene. The Alpha ID screening test utilizes a noninvasive cheek swab screen. If a positive result is found using this test, a follow-up test, Alpha ID Confirm, uses a finger stick and a blood spot card to asses A1AT protein levels as well a potential reflex to next-generation sequencing (NGS) to help physicians achieve an accurate diagnosis of Alpha-1 antitrypsin deficiency (A1ATD) (AlphaID, 2024).

Clinical Utility and Validity

The literature on the analytic and clinical validity of genetic testing for AATD is limited. In addition, few randomized controlled trials (RCTs) have evaluated the impact of AATD testing on patient outcomes. Current evidence-based guidelines (GOLD, 2024) for diagnosis and management of AATD recommend specific interventions for patients with emphysema and AATD. AAT augmentation therapy is often prescribed for patients with AATD and COPD. In addition, several studies have documented that the disease is under-recognized with delay in diagnosis of between five to eight years (Barrecheguren et al., 2016; Stoller et al., 2005).

Snyder et al. (2006) evaluated the laboratory methods of assessing AATD. Samples from 512 individuals were analyzed, and "A1AT concentrations were measured by nephelometry. Phenotype analysis was performed by isoelectric focusing electrophoresis. The genotype assay detected the S and Z deficiency alleles by a melting curve analysis." Of these 512 samples, 10 (2%) were discordant between genotype and phenotype. Of these 10 results, seven were attributed to phenotyping errors. Four percent of the samples submitted to genotype and quantitative analysis were "reflexed" to phenotyping, where phenotyping confirmed the genotype result 85% of the time. The investigators concluded, "The combination of genotyping and quantification, with a reflex to phenotyping, is the optimal strategy for the laboratory evaluation of A1AT deficiency" (Snyder et al., 2006).

Sorroche et al. (2015) examined a cohort of COPD patients and the prevalence of severe AATD. A total of 1002 patients were evaluated, and 785 (78.34%) had normal AAT levels. The remaining 217 patients had low AAT levels, but only 15 patients had a genotype associated with severe AATD. Of these 15 patients, 12 were ZZ and three were SZ. Of the 202 other patients, 29 were a Z heterozygote, 25 were an S heterozygote, and four were an SS homozygote. Lastly, 144 patients could not be definitively diagnosed (Sorroche et al., 2015).

Corda et al. (2011) examined the prevalence of AATD in a supposed "high-risk" area. A total of 817 residents participated, and 67 had low AAT serum levels. Overall, 118 residents carried AATD-related alleles, 114 of which were heterozygotes "(46 Z, 52 S, 9 P(brescia), 4 M(wurzburg), 2 I, 1 P(lowell)". The authors concluded, "the large number of mostly asymptomatic individuals with AATD identified suggests

that in high-risk areas adult population screening programs employing the latest genetic methods are feasible" (Corda et al., 2011).

Soriano et al. (2018) evaluated the prevalence of AATD testing in COPD patients. The patient sample came from "550 UK Optimum Patient Care Research Database general practices". Out of 107,024 COPD patients, only 2.2% had any record of being tested for AATD. Of those tested, 23.7% were diagnosed with AATD. The investigators also noted that between 1994 and 2013, the incidence of AATD diagnosis increased. The authors concluded "that AATD remains markedly underdiagnosed in COPD patients" (Soriano et al., 2018).

Greulich et al. (2016) evaluated the results of a large, targeted screening program for AATD. The samples were distributed by a German AAT laboratory over a period of 12 years, and 18,638 testing kits were obtained. Of this sample, 6919 carried at least one mutation, and 1835 patients were considered to have severe AATD. Overall, 194 of these patients had "rare" genotypes. The authors concluded that "among clinical characteristics, a history of COPD, emphysema, and bronchiectasis were significant predictors for Pi*ZZ, whereas a history of asthma, cough and phlegm were predictors of not carrying the genotype Pi*ZZ" (Greulich et al., 2016).

Mattman et al. (2020) compared the comprehensiveness and efficiency of pathogenic variant (PV) detection of four different protocols from 2011 to 2018 in laboratories across Canada. From 5399 index patients, 396 ZZ genotypes were identified. The protocol for serum A1AT concentration/DNA sequencing in the Ontario center (ON-CD) yielded the highest PV detection – "genotypes with at least one PV, other than S, Z, or F, were identified at 0.67/ZZ as compared to <0.2/ZZ (all others)." However, it also had the highest rates of undefined molecular variants (UMV) (0.16/ZZ vs <0.12/ZZ) or likely benign variants (LBV) compared to all others (0.08/ZZ vs <0.06/ZZ). The authors concluded the "strategies with readily detect variants across the full coding sequence of *SERPINA1* detect more PV as well as more UMV and LBV" (Mattman et al., 2020).

Hamesch et al. (2019) evaluated the clinical landscape of liver symptoms in patients with AATD, specifically the Pi*ZZ genotype. A total of 554 patients (403 exploratory cohort, 151 confirmatory cohort) were included and were compared to 234 controls without pre-existing liver disease. The authors found significantly higher levels of serum liver enzymes in the Pi*ZZ carriers compared to controls, further noting that "significant' fibrosis was suspected in 20%-36% of Pi*ZZ carriers. Signs of advanced fibrosis were 9 to 20 times more common in carriers compared to non-carriers. Controlled attenuation parameter of ≥280 dB/m, which suggests "severe" steatosis was detected in 39% of carriers compared to 31% of controls. Finally, Pi*ZZ carriers were found to have lower serum concentrations of triglyceride, low, and very-low density lipoprotein cholesterol compared to controls, which the authors suggested to represent impaired hepatic secretion of liquid. Overall, the authors concluded that they identified evidence of liver steatosis, impaired liver secretion, liver fibrosis, and that their data could assist in hepatologic management of Pi*ZZ carriers (Hamesch et al., 2019).

Strnad et al. (2019) investigated the impact of the Pi*Z and Pi*S genotypes on subjects with non-alcoholic fatty liver disease (NAFLD) or alcohol misuse. Separate cohorts of 1184 with NAFLD and 2462 with chronic alcohol abuse were included. The authors found Pi*Z genotypes in 13.8% of patients with cirrhotic NAFLD but only 2.4% of patients without liver fibrosis. From there, the increased risk of NAFLD subjects to develop cirrhosis was found to be 7.3 times higher in Pi*Z carriers. The Pi*Z variant was also found in 6.2% of alcohol abusers but only 2.2% of alcohol abusers without significant liver injury. The increased risk was found to be 5.2 times higher in Pi*Z carriers. The Pi*S variant was not associated with NAFLD-related cirrhosis and only mildly with alcohol-related cirrhosis (increased risk = 1.47 times). The authors concluded that the Pi*Z variant was the strongest "single nucleotide polymorphism-based risk

factor for cirrhosis in NAFLD and alcohol misuse, whereas the Pi*S variant confers only a weak risk in alcohol misusers" and remarked that this finding should be considered in future genetic counseling of affected individuals (Strnad et al., 2019).

Carreto et al. (2020) examined the utility of routine screening for AATD among patients with bronchiectasis, due to the contradiction in guidelines from the British Thoracic Society, which recommend screening for bronchiectasis among patients with AATD, but not vice versa. After screening 1600 patients with bronchiectasis from two centers in the UK from 2012-2016, they found only eight patients with AATD. They concluded that because of the low prevalence of AATD as an etiology for disease presentation among patients with bronchiectasis, routine screening for AATD would not significantly impact clinical management through augmentation therapy, smoking cessation, and genetic counselling, among other methods. Despite this, the researchers did note that higher rates of detection may be found in other geographical regions in the UK or in other countries (Carreto et al., 2020).

In 2021, Murray et al. evaluated the efficacy of a liquid chromatography-tandem mass spectrometry (LC-MS/MS) (proteotyping)-based algorithm for AATD detection (n=5474), as compared to the more traditional isoelectric focusing (IEF) phenotyping (n=16147). Here, the authors found that LC-MS/MS reduced the rate of IEF) phenotyping by 97% and the 3% of cases that were reflexed to IEF resulted in and addition 0.2% of phenotype findings. By retrospectively applying the proteotype-based algorithm to the IEF cohort, they demonstrated a 99.9% sensitivity for the detection of deficiency-associated phenotypes. The authors concluded that the "proteotype algorithm is a sensitive and cost-effective approach for the diagnosis of clinical AAT deficiency" (Murray et al., 2021).

Bellemare et al. (2021) studied the clinical utility of determining the allelic background of mutations causing alpha-1 antitrypsin deficiency. *SERPINA1* was DNA sequenced to identify rare variants that could confer the risk of developing emphysema. Seven carriers of a rare variant, Leu353Phe_fsTer24, known to lead to undetectable serum levels of AAT, were studied using an allele-specific DNA sequencing method that they developed. Results demonstrated that Leu353Phe_fsTer24 variant was transmitted on the same allele as the M3 variant in all the patients and two of the seven patients had either a S or Z allele. The lowest AAT serum levels were observed in compound heterozygotes for the S or Z allele, suggesting higher risk of developing emphysema. This study showed that understanding the clinical significance of genetic variants found in SERPINA1 can lead to better clinical outcomes (Bellemare et al., 2021).

Ashenhurst et al. (2022) conducted a study to examine whether direct-to-consumer genetic testing increased the identification of previously undetected individuals specifically with AATD, and whether it impacted clinical care. In this cross-sectional study using a survey from the 23andMe, Inc. research platform with 195,014 participants, researchers found that the allele frequency of PI*S was 15.1%, 6.5% for PI*Z, and 0.63% with PI*ZZ. Half of those with the PI*ZZ allele combination were able to confirm their diagnosis with a physician. Twenty seven percent of the participants were first made aware of their disease status through this test, and among these participants, "the diagnostic delay interval was 22.3 years." As a result of this finding, there was a 1.7 times increased odds of reporting smoking reduction and 4.0 times increased odds of reporting reduced alcohol consumption. This demonstrates that having convenient methods of detecting pathogenic variants of the *SERPINA1* gene in commercial testing could benefit patients in the long run in terms of reducing risks of complications from AATD (Ashenhurst et al., 2022).

Balcar et al. (2022) studied the association between the alpha-1 antitrypsin Pi∗Z allele and liver disease. The study included 1118 patients with advanced chronic liver disease, all of whom had undergone genotyping for the Pi*Z/Pi*S allele. Compared to non-carriers, Pi∗Z carriers had more severe portal hypertension and hepatic dysfunction. "Harbouring the Pi∗Z allele was significantly associated with an

increased probability of liver transplantation/liver-related death," but "the Pi∗S allele was unrelated to liver disease severity" and "Pi∗S carriers had no increased risk of events." The authors concluded that "genotyping for the Pi∗Z allele identifies patients with ACLD at increased risk of adverse liver-related outcomes, thereby improving prognostication" (Balcar et al., 2022).

Clark et al. (2018) studied the clinical and histologic features of individuals with AATD. The study included 94 non-cirrhotic adults with Pi*ZZ AATD. "The prevalence of clinically significant liver fibrosis (F ≥ 2) was 35.1%. Alanine aminotransferase, aspartate aminotransferase and gamma-glutamyltransferase values were higher in the F ≥ 2 group." Metabolic syndrome, the presence of accumulated abnormal AAT in hepatocytes, portal inflammation, and hepatocellular degeneration were all associated with clinically significant fibrosis. The authors concluded that "over one-third of asymptomatic and lung affected adults with 'PI*ZZ' AATD have significant underlying liver fibrosis" and that "liver disease in this genetic condition may be related to a "toxic gain of function" from accumulation of AAT in hepatocytes" (Clark et al., 2018).

Eriksson et al. (1986) studied the association between AATD and cirrhosis and primary liver cancer. The study included 17 autopsy cases of individuals with AATD. Each autopsy was matched with four control cases. "The results indicated a strong relation between alpha 1-antitrypsin deficiency and cirrhosis and primary liver cancer" (Eriksson et al., 1986).

Fromme et al. (2022) studied the association between AATD and hepatobiliary phenotypes. The study included 1104 participants (586 Pi*ZZ, 239 Pi*SZ, 279 non-carriers). "Pi*ZZ individuals displayed the highest liver enzyme values, the highest occurrence of liver fibrosis/cirrhosis and primary liver cancer… Pi*SZ participants displayed higher liver enzymes, more frequent liver fibrosis/cirrhosis… Subjects with Pi*MZ genotype had slightly elevated liver enzymes and moderately increased odds for liver fibrosis/cirrhosis…Individuals with homozygous Pi*S mutation (Pi*SS genotype) harboured minimally elevated alanine aminotransferase values." The authors concluded that their findings classify hepatobiliary phenotypes with their most relevant AATD genotypes (Fromme et al., 2022).

The Childhood Liver Disease Research Network Longitudinal Observational Study of Genetic Causes of Intrahepatic Cholestasis (Teckman et al., 2020) is a longitudinal study about pediatric cholestatic liver disease. The study included 350 participants ages zero to 25 with native livers. Overall, 18 participants developed hypertension, and two died, but "there was no difference in participants with or without preceding neonatal cholestasis progressing to transplantation or death during the study, or in experiencing portal hypertension." The authors concluded that, in youth with AATD, "progression to liver transplantation is slow and death is rare, but the risk of complications and severe liver disease progression persists throughout childhood." The authors also note that "A history of neonatal cholestasis is a weak predictor of severe disease" (Teckman et al., 2020). Teckman et al. (2023) then focused on neonatal cholestasis in children with AATD, using two subgroups: participants with neonatal cholestasis (n=46), and all participants who progressed to livre transplant (n=119). The authors reported "an association of neonatal gamma-glutamyl transpeptidase elevation to more severe disease, and a higher rate of neonatal cholestasis progression to portal hypertension than previously reported (41%) occurring at median age of 5 months." All participants, regardless of neonatal cholestasis, were at risk of progression to liver transplant, but of the participants that progressed to liver transplant, those with neonatal cholestasis were significantly younger at transplant than those without neonatal cholestasis. The authors further concluded that "patients with AATD and neonatal cholestasis are at risk of early progression to severe liver disease, but the risk of severe disease extends throughout childhood" (Teckman et al., 2023).

Lin et al. (2019) completed a systematic literature review of AATD deficiency-associated liver disease in children and put together diagnostic testing recommendations. The literature review revealed that "liver disease occurs in 10% of children, manifested by cholestasis, pruritus, poor feeding, hepatomegaly, and splenomegaly, but the presentation is highly variable." The authors recommend genetic testing for AATD in children with unexplained liver disease or suspected AATD, noting "consensus guidelines recommend diagnostic testing for all patients who have unexplained liver disease" (level of evidence for recommendations: A, based on consistent and good quality patient-oriented evidence) (Lin et al., 2019).

Guidelines and Recommendations

American Thoracic Society/European Respiratory Society (ATS/ERS)

The ATS/ERS released joint guidelines on the "Diagnosis and Management of Individuals with Alpha-1 Antitrypsin Deficiency." These recommendations are as follows (ATS/ERS, 2003):

Policy Guidelines

Recommendations were classified as follows:

Type A: Genetic testing is recommended

Type B: Genetic testing should be discussed and could be accepted or declined

Type C: Genetic testing is not recommended, i.e., should not be encouraged

Type D: Recommend against genetic testing, i.e., should be discouraged

Type A recommendations for diagnostic testing in the following situations:

1. Symptomatic adults with emphysema, COPD or asthma with airflow obstruction that is not completely reversible with aggressive treatment with bronchodilators
2. Individuals with unexplained liver disease, including neonates, children, and adults, particularly the elderly
3. Asymptomatic individuals with persistent obstruction on pulmonary function tests with identifiable risk factors (e.g., cigarette smoking, occupational exposure)
4. Adults with necrotizing panniculitis
5. Siblings of an individual with known alpha-1 antitrypsin (AAT) deficiency

Type B recommendations for diagnostic testing in the following situations:

1. Adults with bronchiectasis without evidence etiology
2. Adolescents with persistent airflow obstruction
3. Asymptomatic individuals with persistent airflow obstruction and no risk factors
4. Adults with C-ANCA positive (anti-proteinase 3-positive) vasculitis
5. Individuals with a family history of COPD or liver disease not known to be attributed to AAT deficiency
6. Distant relatives of an individual who is homozygous for AAT deficiency
7. Offspring or parents of an individual with homozygous AAT deficiency
8. Siblings, offspring, parents or distant relatives of an individual who is heterozygous for AAT deficiency
9. Individuals at high risk of having AAT deficiency-related diseases

10. Individuals who are not at risk themselves of having AAT deficiency but who are partners of individuals who are homozygous or heterozygous for AAT deficiency

Type C recommendations for diagnostic testing in the following situations

1. Adults with asthma in whom airflow obstruction is completely reversible
2. Predispositional testing
3. Population screening of smokers with normal spirometry

Type D recommendations for diagnostic testing in the following situations:

1. Predispositional fetal testing
2. Population screening of either neonates, adolescents or adults*

* Population screening is not recommended currently. However, a possible exception (type B recommendation) may apply in countries satisfying all three of the following conditions: (1) the prevalence of AAT deficiency is high (about 1/1,500, or more); (2) smoking is prevalent; and (3) adequate counseling services are available.

The following features should prompt suspicion by physicians that their patient may be more likely to have AAT deficiency:

Clinical Factors

- Early-onset emphysema (age of 45 years or less)
- Emphysema in the absence of a recognized risk factor (smoking, occupational dust exposure, etc.)
- Emphysema with prominent basilar hyperlucency
- Otherwise unexplained liver disease
- Necrotizing panniculitis
- Anti-proteinase three-positive vasculitis (C-ANCA [anti-neutrophil cytoplasmic antibody]-positive vasculitis)
- Bronchiectasis without evident etiology

The ATS/ERS also made statements on serum testing for AATD. "Serum phenotyping by isoelectric focusing performed by a reliable laboratory is the accepted "gold standard" for diagnosing AAT deficiency". The guidelines recommend "that all subjects with COPD or asthma characterized by incompletely reversible airflow obstruction should be tested once for quantitative AAT determination. Also, individuals with evidence of cirrhosis of the liver with no known etiology should be tested for candidate phenotypes (e.g., PI*ZZ, PI*MZ, PI*Mmalton) and testing should be considered in individuals with the syndrome of Wegener's granulomatosis (antiproteinase-3 vasculitis)" (ATS/ERS, 2003).

The ATS/ERS states that "Regarding hepatic presentations of AAT deficiency later in childhood, during adolescence, and in adulthood, reports indicate that patients may present with hepatosplenomegaly, ascites, upper gastrointestinal bleeding resulting from esophageal varices, chronic hepatitis, cirrhosis, or hepatic failure. The presentation of AAT deficiency may appear similar to other chronic liver diseases, including autoimmune hepatitis, drug-induced hepatitis, chronic viral hepatitis, and Wilson's disease. The weight of these reports suggests that patients with any unexplained features of chronic liver disease should be evaluated for AAT deficiency" (ATS/ERS, 2003).

American College of Gastroenterology (ACG)

The ACG notes that "defective production of the alpha-1 anti-trypsin protein may result in both panacinar emphysema and chronic obstructive pulmonary disease, as well as progressive liver disease, liver cirrhosis, and hepatocellular carcinoma" and recommends the following for AATD:

- "Patients with persistently elevated aspartate aminotransferase (AST) or alanine aminotransferase (ALT) should undergo screening for alpha-1 antitrypsin (A1AT) deficiency with alpha-1 anti-trypsin phenotype."
- Evaluation of hepatocellular injury (defined by the guidelines as "disproportionate elevation of AST and ALT levels compared with alkaline phosphatase levels") includes testing for A1AT deficiency" (Kwo et al., 2017).

National Organization for Rare Disorders

NORD publishes information on multiple rare disorders that may otherwise lack national guidelines and recommendations. One such disorder is neonatal cholestasis, which refers to impaired flow of bile from the liver cells into the intestine of a newborn. While neonatal cholestasis may be caused by viruses, metabolic disease, or rare disease that affect or impair the function of the liver, it can also be caused by genetic disorders. "The incidence of neonatal cholestasis is estimated to be ~1:2500 live births worldwide, and 25% to 50% are now known to be associated with changes (variants or mutations) in specific genes" (NORD, 2024b). These genetic disorders include "alpha-1-antitrypsin deficiency, PFIC and Alagille syndrome" (NORD, 2024b).

World Health Organization (WHO)

The WHO released a memorandum on AATD regarding AATD's association with conditions such as COPD and asthma. Their recommendation is as follows: "It is therefore recommended that all patients with COPD and adults and adolescents with asthma be screened once for AAT deficiency using a quantitative test. Those with abnormal results on screening should undergo PI typing" (WHO, 1997).

European Respiratory Society (ERS)

The ERS (Miravitlles et al., 2017) published updated guidelines which recommend:

- "The quantitative determination of AAT levels in blood is a crucial first test to identify AATD. Quantitative deficiency must be supported by qualitative tests to identify the genetic mutation(s) causing AATD."
- "Protein phenotyping by isoelectric focusing identifies variants where AAT is present in the sample including the rarer variants F, I and P *etc*."
- "Genotyping allows a rapid and precise identification/exclusion of S and Z alleles and other variants, where specific primers are available."
- "Gene sequencing remains necessary for those cases where a null variant or a deficient variant other than Z or S is suspected."
- "Testing of relatives of identified patients should be considered after appropriate counselling.
- "Genetic testing should be carried out only after informed consent is given and in accordance with the relevant guidelines and legislation."

The ERS has also noted that "there is no evidence to support efficacy of AAT augmentation therapy in PiSZ, PiMZ or current smokers of any protein phenotype" (Miravitlles et al., 2017).

Alpha-1 Foundation

The Alpha-1 Foundation (Sandhaus et al., 2016) sponsored a medical and scientific advisory committee of experts to examine all relevant, recent literature to provide concise recommendations for the diagnosis and management of individuals with AATD.

- "For family testing after a proband is identified, AAT level testing alone is not recommended because it does not fully characterize disease risk from AATD."
- "For diagnostic testing of symptomatic individuals, they recommend genotyping for at least the S and Z alleles. Advanced or confirmatory testing should include Pi-typing, AAT level testing, and/or expanded genotyping."
- "All patients with COPD, unexplained chronic liver disease, necrotizing panniculitis, granulomatosis with polyangiitis, or unexplained bronchiectasis should be tested for AATD."
- "Parents, siblings, and children, as well as extended family of individuals identified with an abnormal gene for AAT, should be provided genetic counseling and offered testing for AATD (see guideline document for special considerations about testing minors)."

The Foundation also noted the following (these statements were not labeled recommendations):

- "For primary diagnosis of AATD the most sensitive and specific method of diagnosis is direct identification of the Z allele by genotyping. By also including the S allele, genotyping for the S and Z allele is greater than 99% specific and sensitive. "
- "AAT levels are insufficient to identify at risk individuals because the AAT level changes with inflammation, pregnancy, and in children. "
- "The range of serum AAT levels among individuals with specific genotypes is sufficiently broad that there is overlap between different genotypes. Thus, serum AAT levels cannot discriminate between different genotypes and additional AAT testing is needed" (Sandhaus et al., 2016).

Global Initiative for Chronic Obstructive Lung Disease (GOLD)

The GOLD guideline notes that "The most relevant (albeit rare) genetic risk factor for COPD identified to date are mutations in *SERPINA1* gene leading to α-1 antitrypsin deficiency. A number of other genetic variables have also been associated with reduced lung function and risk of COPD, but their individual effect size is small."(GOLD, 2024).

Canadian Thoracic Society (CTS)

The CTS released guidelines on genetic testing for AATD, which are as follows:

- "We suggest targeted testing for A1AT deficiency be considered in individuals with COPD diagnosed before 65 years of age or with a smoking history of <20 pack years. (Grade of recommendation: 2C)"
- "We suggest targeted testing for A1AT deficiency not be undertaken in individuals with bronchiectasis or asthma. (Grade of recommendation: 2C)" (Marciniuk et al., 2012)

National Institute Health and Care Excellence (NICE)

NICE published a guideline discussing chronic obstructive pulmonary disease (COPD) in 2019. In it, they note that measurement of serum alpha-1 antitrypsin has a role in identifying deficiencies if the condition is "early onset, [of] minimal smoking history, or [has] family history" (NICE, 2019).

Government of British Columbia

The British Columbia guidelines on the diagnosis and management of COPD state that, "testing for A1AT deficiency is expensive, low yield, often duplicated and may not alter management in a meaningful way. Therefore, refer patients with high pre-test probability to a specialist" (BC Guidelines, 2024).

Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: http://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration

On November 17, 2017, the FDA approved Grifols' (Grifols, 2017) *SERPINA1* Variant Detection System as a qualitative in vitro molecular diagnostic system used to detect variants in *SERPINA1* gene in genomic DNA isolated from human specimens. On November 7, 2019, the FDA approved Grifols' AlphaID™, a cheek swab that can screen patients with COPD for alpha-1 antitrypsin deficiency. It "utilizes an FDA-approved genotyping assay to screen for the 14 most prevalently reported genetic mutations associated with Alpha-1, including the S, Z, F, I alleles, as well as rare and null alleles, helping detect patients who are at risk for this treatable condition" (Grifols, 2019).

On April 6, 2017 the FDA approved (FDA, 2017) the 23andMe PGS Genetic Health Risk Report for Alpha-1 Antitrypsin Deficiency (AATD) which determines if a person has variants associated with a higher risk of developing AATD-associated lung or liver disease. This report is based on a qualitative genetic test for single nucleotide polymorphism detection of the PI*Z (rs28929474) and PI*S (rs17580) variants in the *SERPINA1* gene by using the 23andMe Personal Genome Service.

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
82103	Alpha-1-antitrypsin; total
82104	Alpha-1-antitrypsin; phenotype
82542	Column chromatography, includes mass spectrometry, if performed (eg, HPLC, LC, LC/MS, LC/MS-MS, GC, GC/MS-MS, GC/MS, HPLC/MS), non-drug analyte(s) not elsewhere specified, qualitative or quantitative, each specimen
83789	Mass spectrometry and tandem mass spectrometry (eg, MS, MS/MS, MALDI, MS-TOF, QTOF), non-drug analyte(s) not elsewhere specified, qualitative or quantitative, each specimen

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

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Revision History

Revision Date	Summary of Changes

09/04/2024	Reviewed and Updated: Updated background, guidelines, and evidence-based scientific references. Literature review necessitated the following changes in coverage criteria: Addition of "once per lifetime" to CC1. Now reads: "1) For individuals who are suspected of having alpha-1 antitrypsin (AAT) deficiency, serum quantification of alpha-1 antitrypsin (AAT) protein and AAT phenotyping or AAT proteotyping (see Note 1) MEETS COVERAGE CRITERIA once per lifetime in any of the following situations:" Edited CC1.b. to include examples of unexplained liver disease. Now reads: "b) For individuals with unexplained liver disease (e.g., chronic hepatitis with or without cirrhosis, chronically elevated aminotransferase levels, portal hypertension, primary liver cancer)." New CC1h: "h) For individuals with neonatal cholestasis."

Testing for Developmental Delay

Policy Number: AHS – M2176 – Testing for Developmental Delay	Initial Presentation Date: 12/02/2020 Effective Date: 4/1/2025

Policy Description

Autism spectrum disorder (ASD) is a complex condition typically associated with deficits in social interaction and communication, as well as restrictive and repetitive behaviors and sensory issues (Ivanov et al., 2015; Persico et al., 2019). ASD is typically identified in early childhood (Lord et al., 2018; Persico et al., 2019) and has multiple etiologies, subtypes, and developmental trajectories (Masi et al., 2017). Intellectual disability, attention deficit hyperactivity disorder, and epilepsy are commonly seen in children with ASD (Augustyn, 2024). Further, ASD is known to have a strong genetic component and is diagnosed in all racial, ethnic, and socioeconomic groups (Ivanov et al., 2015; Lord et al., 2018).

For individuals without signs of syndromic developmental delay or a metabolic disorder causing developmental delay, please see guidance on chromosomal microarray testing (AHS-M2033-Chromosomal Microarray) and whole exome sequencing (AHS-M2032-Whole Genome and Whole Exome Sequencing). For guidance regarding testing for *FMR1* mutations or Rett syndrome, please refer to AHS-M2028-Genetic Testing for *FMR1* Mutations and AHS-M2088-Genetic Testing for Rett Syndrome, respectively.

Related Policies

Policy Number	Policy Title
AHS-M2028	Genetic Testing for *FMR1* Mutations
AHS-M2032	Whole Genome and Whole Exome Sequencing

AHS-M2033	Chromosomal Microarray and Low-pass Whole Genome Sequencing
AHS-M2070	Genetic Testing for CHARGE Syndrome
AHS-M2075	Genetic Testing for Epilepsy
AHS-M2088	Genetic Testing for Rett Syndrome

Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) For individuals less than 18 years of age who have had a physical examination suggestive of syndromic developmental delay or developmental delay due to a metabolic disorder (e.g., dysmorphology, growth parameters [including head circumference], skin examination), targeted genetic testing **MEETS COVERAGE CRITERIA**.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

2) For the diagnosis of autism spectrum disorder (ASD) or non-syndromic developmental delay, all other testing outside of chromosomal microarray, whole exome sequencing, or whole genome sequencing or genetic testing for fragile X syndrome or Rett syndrome **DOES NOT MEET COVERAGE CRITERIA**.

NOTES:

Note: For two or more gene tests being run on the same platform, please refer to AHS-R2162 Reimbursement Policy.

Table of Terminology

Term	Definition
AACAP	American Academy of Child and Adolescent Psychiatry
AAP	American Academy of Pediatrics
ACMG	American College of Medical Genetics and Genomics
ADHD	Attention deficit hyperactivity disorder
ADOS-2	Autism diagnostic observation schedule
ASD	Autism spectrum disorder
AUC	Area under the curve
CAMP	Children's Autism Metabolome Project
CDC	Centers for Disease Control and Prevention
CLIA '88	Clinical Laboratory Improvement Amendments of 1988
CMA	Chromosomal microarray
CMS	Centers for Medicare and Medicaid Services

CNS	Central nervous system
CNVs	Copy number variants
DD	Developmental disability
DISCO	Diagnostic interview for social and communication disorders
DSM	Diagnostic and Statistical Manual of Mental Disorders
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition
FASD	Fetal alcohol spectrum disorder
FDA	Food and Drug Administration
FMR1	*Fragile X messenger ribonucleoprotein 1*
ID	Intellectual disability
IQ	Intelligence quotient
ISCA	International standard cytogenomic array
LC-MS/MS	Liquid chromatography–mass spectrometry/mass spectrometry
LDH	Lactate dehydrogenase
LDT	Laboratory-developed test
LKS	Landau-Kleffner syndrome
MCA	Multiple congenital anomalies
M-CHAT	Modified Checklist for Autism in Toddlers
M-CHAT-R/F	M-CHAT, revised with follow-up questions
MECP2	*Methyl-CpG binding protein 2*
miRNA	Micro ribonucleic acid
NGS	Next-generation sequencing
NICE	National Institute for Health and Care Excellence
NIMH	National Institutes of Mental Health
OCD	Obsessive-compulsive disorder
PRS	Polygenic risk scores
PTEN	*Phosphatase and tensin homolog*
RNA	Ribonucleic acid
rRNA	Ribosomal ribonucleic acid
SCQ	Social communication questionnaire
SCZ	Schizophrenia
SDs	Standard deviations
SNP	Single-nucleotide polymorphisms
SRS	Social responsiveness scale
TD	Typical development
USPSTF	United States Preventive Services Task Force
WES	Whole exome sequencing

Scientific Background

Autism spectrum disorder (ASD) is a prevalent neurodevelopmental disorder affecting approximately one in 36 children in the United States (CDC, 2024b). ASD is typically characterized by impaired social interaction and other restrictive and repetitive behaviors, and ASD is diagnosed behaviorally based on the presence of abnormal social communication and repetitive behavior (Vuong & Hsiao, 2017).

The condition may be either idiopathic or syndromic, with many syndromic cases related to genetic disorders, such as Rett syndrome (Persico et al., 2019). While many aspects relating to the etiology of the condition are poorly understood, ASD is known to have a strong genetic component; many familial inheritance patterns have been associated with the condition, and up to 1000 genes may be potentially implicated (Ramaswami & Geschwind, 2018). Known ASD risk factors include chromosomal deletion(s) and prematurity (Muhle et al., 2018). Researchers report that parents who have a child with autism have a 2-18% chance of having another child with autism (Lyall et al., 2017; Waye & Cheng, 2018).

Diagnosis of ASD includes a comprehensive evaluation by a multidisciplinary team to determine if the child's symptoms meet the criteria for ASD, as outlined in the *Diagnostic and Statistical Manual of Mental Disorders* (DSM), to determine the child's neurodevelopment profile of strengths and weaknesses, and to assess whether the child has any other associated or underlying condition(s) (Augustyn & von Hahn, 2024). The fifth edition of the DSM requires the individual have all the following:

- Social communication and social interaction deficits in multiple settings as demonstrated by all of the following—social-emotional reciprocity, a lack of understanding or awareness of the feelings of others; nonverbal communicative behaviors; difficulty in developing, maintaining, and understanding interpersonal relationships; and
- Repetitive behavior patterns as demonstrated with at least two of the following—stereotyped or repetitive movements; obsessive, compulsive adherence to routines or patterns of verbal or nonverbal behaviors; highly fixated, abnormal preoccupation on specific interests; increased or decreased responses to sensory input, such as adverse responses to environment; and
- Impaired function due to symptoms; and
- Symptoms present early in developmental period of life; and
- Symptoms "are not better explained by intellectual disability (formerly referred to as mental retardation) or global developmental delay" (Augustyn & von Hahn, 2024).

A differential diagnosis of ASD from other conditions that impair social communication, interaction, or development may help guide possible therapeutic options. These conditions include both global delay (or intellectual disability) and intellectual giftedness, social communication disorder, developmental language disorder, learning disorders, hearing impairment, Landau-Kleffner syndrome (LKS or acquired epileptic aphasia), Rett syndrome, fetal alcohol spectrum disorder (FASD), attachment disorder, attention deficit hyperactivity disorder (ADHD), anxiety disorder, obsessive-compulsive disorder (OCD), stereotypic movement disorder, or tic disorders (such as Tourette syndrome). Each of these disorders may share individual characteristics of ASD while exhibiting distinguishing characteristics for their respective condition, such as appropriate imaginative play, normal reciprocal social interactions, or specific morphologies (e.g. the characteristic facial features of FASD) (Augustyn & von Hahn, 2024; Hyman et al., 2020; Volkmar et al., 2014).

Both genetic and environmental factors play a part in the etiology of ASD. Even though ASD is more prevalent in males, male-to-male transmission in certain lineages indicates that ASD is not solely X-linked (Muhle et al., 2004). Genetic analysis can be performed using chromosomal microarray, karyotype, or genetic sequencing including next-generation sequencing (NGS) or whole exome sequence (WES) analysis. Specific genetic testing should be based on the clinical findings of the affected individual and family history. The American College of Medical Genetics and Genomics reports "the following approximate diagnostic yields are expected in the genetic evaluation of ASDs:

- CMA [chromosomal microarray] (10%)
- Fragile X (1-5%)

- *MECP2* (4% of females)
- *PTEN* (5% of those with head circumferences >2.5 SDs [standard deviations] that are tested)
- Karyotype (3%)
- Other (10%). Currently, there are no published studies that collate the yield on the other identifiable etiologies of autism… Using empiric estimates and clinical experience, this has been estimated as 10%" (Schaefer & Mendelsohn, 2013).

Chromosomal microarray (CMA) is a microarray-based genomic copy number analysis test that can be used to help diagnose unexplained developmental delay, intellectual disability, and ASD, as well as multiple congenital anomalies (Miller et al., 2010). CMA cannot detect balanced translocations; however, these de novo translocations are infrequently encountered (Beaudet, 2013). Karyotype can be used in instances where a balanced translocation is suspected, such as a history of two or more miscarriages (Augustyn & von Hahn, 2024).

Besides CMA and karyotyping, genetic sequencing can be used to screen for possible mutations, specifically NGS and WES analysis. NGS refers to the use of a single platform to sequence multiple strands of nucleic acid rapidly in parallel. This technique can be utilized to screen the entire exome for WES analysis (Hulick, 2024). A number of genetic tests are commercially available, including the Clarifi™ test (Quadrant Biosciences, Inc.), an NGS-based saliva test that measures epigenetic microRNAs and the microbiome to generate an algorithmic report of the predictive probability of ASD (Quadrant Biosciences, 2024).

While it is believed that inborn errors of metabolism account for only 5% of autistic individuals, defects in one-carbon metabolism are one of the most-often reported physiopathologies reported to be associated with autism, as these disturbances to cellular bioenergetics lead to increased oxidative stress, impaired redox homeostasis, and methylation disruption, the third of which portend to deficits in gene expression, neurotransmitter synthesis, and neuronal synchronization (Carrasco et al., 2019; Paşca et al., 2009). Similarly, with regard to amines, neurotransmitters that are amino acids and their derivatives are thought to play critical roles in the diagnosis of ASD, with hypotheses ranging from the differentiation and migration of neurons, the synaptic plasticity of neurons, and the perturbation of reward- and motivation-related circuits modulated by concentrations of serotonin, glutamate, and dopamine, respectively (Pavăl, 2017; Vargason et al., 2018). It is no surprise, then, that the richness of the field means that it is ripe for a foray into expanding our understanding of ASD and the concomitant therapeutic consequences.

In addition to genetic testing, different biomarkers have been proposed as possible aids in diagnosing ASD and developmental delay. The Children's Autism Metabolome Project (CAMP) [Clinical Trials Identifier NCT02548442], funded by a grant from the National Institutes of Mental Health (NIMH) and sponsored by Stemina Biomarker Discovery, Inc., has a goal to identify metabolic signature profiles in blood plasma and/or urine that can differentiate children with ASD from children with either non-ASD delayed development or typical development. CAMP, expected to be completed in 2023, is a multi-center clinical study at eight sites within the United States that has more than 1100 enrolled participants as of 2020 (NeuroPointDx, 2024a; NLM, 2020). To date, the focus has been on amino acid metabolism dysregulation. Stemina Biomarker Discovery, Inc. does offer commercially available biomarker tests for ASD, including the NeuroPointDX Autism Spectrum Disorder (NPDX ASD) blood test, that measures 32 different amines present in blood plasma using LC-MS/MS. Then, a proprietary algorithm based on the ADOS-2 reference method indicates the individual's metabolic subtype (or "metabotype"). For example, in a positive metabotype three profile "indicate[s] a positive metabolic profile associated with ASD. The

imbalance detected indicates an increase in concentration of Glycine relative to the concentration of Asparagine" (NeuroPointDx, 2024b).

Xiaoxiao et al. (2023) conducted a plasma proteomic and metabolomic study of 122 children with ASD. Participants were divided into three groups: a group of children that had risk genes (de novo mutations), ASD children without risk genes, and a healthy control group. After plasma proteomics and metabolomics analyses, the authors found "the protein or metabolism profile of the children with or with no risk genes was more clustered and overlapped, with a separation trend from the control." In ASD children, most complement pathway proteins were upregulated, and the authors noted specifically the complement pathway proteins: "C2, CPB2, IGHV3-74, and IGHV5051," were upregulated, "supporting the notion that autistic patients may experience complement activation in their peripherals" (Xiaoxiao et al., 2023).

Clinical Utility and Validity

Tammimies et al. (2015) compared the molecular diagnostic yield of CMA and WES in children (n = 258) with ASD, split into three groups based on severity of morphology (essential, equivocal, and complex). They note that 15.8% of the children who underwent both CMA and WES testing have an identifiable genetic etiology. Statistical differences between the three morphological groups were recorded, and the "combined yield was significantly higher in the complex group when compared with the essential group (pairwise comparison, P = .002)." Individually, CMA and WES produced similar yields; for example, 4.2% of children in the essential group tested positive with CMA as compared to 3.1% undergoing WES. The authors conclude that "the molecular diagnostic yields of CMA and WES were comparable… If replicated in additional populations, these findings may inform appropriate selection of molecular diagnostic testing for children affected by ASD" (Tammimies et al., 2015). Rossi et al. (2017) performed a study of WES on 163 individuals with either ASD or autistic features, reporting that 61.9% of positive findings were *de novo* mutations. Moreover, individuals "presenting with psychiatric conditions or ataxia or paraplegia in addition to autism spectrum disorder or autistic features were significantly more likely to receive positive results compared with patients without these clinical features (95.6% vs 27.1%, $P < 0.0001$; 83.3% vs 21.2%, $P < 0.0001$, respectively)" (Rossi et al., 2017).

Ragusa et al. (2020) evaluated 53 ASD children who were treatment-naïve and 27 unaffected controls by performing miRNA expression profiling and 16S rRNA microbiome analysis on saliva samples. Their results show an upregulation of miR-29a-3p and miR-141-3p and downregulation of miR-16-5p, miR-let-7b-50, and mi-R-451a in children with ASD. "Microbiome analysis on the same subjects revealed that *Rothia, Filifactor, Actinobacillus, Weeksellaceae, Ralstonia, Pasteurellaceae*, and *Aggregatibacter* increased their abundance in ASD patients, while *Tannerella, Moryella* and *TM7-3* decreased" (Ragusa et al., 2020).

Hicks et al. (2018) performed a multi-center, cross-sectional validation study of the Clarifi™ test (Quadrant Biosciences, Inc.) using children aged 19 to 83 months. The individuals were divided among three groups: a control, neurotypical group (n=134); a group with a diagnosis of ASD (n=238); and a group with non-ASD developmental delay (n=84). Randomly, prior to initiating the study, all individuals were further divided between the training and independent validation sets (82% and 18%, respectively). The training set established the RNA-based algorithm to be used to distinguish ASD and non-ASD children while the validation set was used to test the algorithm accuracy. Using the established algorithm from the training set, the authors state that the validation test "maintained an AUC of 0.88 (82% sensitivity and 88% specificity). Notably, the RNA features were implicated in physiologic processes related to ASD (axon guidance, neurotrophic signaling)" (Hicks et al., 2018). These data further supported their earlier findings from a smaller study (n=45) that 14 miRNAs "were differentially

expressed in ASD subjects compared to controls ($p < 0.05$; FDR <0.15) and showed more than 95 % accuracy at distinguishing subject groups in the best-fit logistic regression model" (Hicks et al., 2016).

Hicks et al. (2020) also performed a multi-center study to use saliva microRNAs to differentiate children with ASD (n=187) from peers with typical (n=125) or non-ASD atypical development (n=69). In total, 14 miRNAs showed differential expression, and four miRNAs "best differentiated children with ASD from children without ASD in training (area under the curve = 0.725) and validation (area under the curve = 0.694) sets. Eight microRNAs were associated ($R > 0.25$, false discovery rate < 0.05) with social affect, and 10 microRNAs were associated with restricted/repetitive behavior." The authors conclude, "Salivary microRNAs are "altered" in children with ASD and associated with levels of ASD behaviors. Salivary microRNA collection is noninvasive, identifying ASD-status with moderate accuracy. A multi-'omic' approach using additional RNA families could improve accuracy, leading to clinical application" (Hicks et al., 2020).

László et al. (1994) reported that not only were the mean values of serotonin for autistic children higher as compared to the control group– 1.253 µmol/l and 0.88 µmol/l, respectively–but also that hyperserotonemia was detected in 20 of the 46 autistic children, corroborating a previous report of elevated levels of serum serotonin in 40% of affected children (László et al., 1994). The study also noted that in 20% (n=30) of patients lactic acidosis and hyperpyruvatemia were detected in the absence of hyperserotonemia, urging that these metabolites be explored as important targets for managing infantile autism (László et al., 1994).

A Seoul National University Bandung Hospital study that recruited 59 subjects with ASD–sorted into the affected group–and their unaffected family members (both biological parents and unaffected siblings), who comprised the unaffected group of 135 members provides evidence for the use of mitochondrial markers in the diagnosis of ASD (Oh et al., 2020). The measuring of carbon metabolites demonstrated that not only did the affected group boast significantly higher lactate than the unaffected group (19.79 ± 11.29 vs. 13.84 ± 6.12 mg/dl at $p<0.01$) but also had higher lactate-to-pyruvate ratios (21.47 ± 18.43 vs. 15.03 ± 9.37 at $p<0.05$); however, there were no significant correlations between the parameters themselves (Oh et al., 2020). This supports previous findings that reported elevated lactate and lactate-to-pyruvate ratios in ASD individuals, and further corroborates the notion that defects in mitochondria can lead to and potentially explain neurodevelopmental disorders for their roles in both aerobic energy production and the development of neurons in the CNS (Oh et al., 2020; Paşca et al., 2009).

A study focusing on the profile of metabolic abnormalities expected due to mitochondrial dysfunction demonstrated that in a sample of 146 Egyptian boys (73 autistic, 73 unaffected), plasma levels of lactate and serum pyruvate, lactate-to-pyruvate ratio, creatine kinase, pyruvate kinase, and LDH (glycolytic enzyme expression) were significantly higher ($p<0.05$) among the subjects than in the unaffected control group, while amines such as serum L-carnitine (participating in the beta-oxidation of fatty acids) and urea were in turn lower, save for ammonia (Hassan et al., 2019). Interestingly, blood levels of all previously measured biochemical markers did not differ between mild to moderately autistic children as compared to those severely impacted except for significantly higher oxidative stress index and significantly lower antioxidant levels, suggesting that the ASD is not explicable by a singular etiology (Hassan et al., 2019).

Although the testing of any one metric for the diagnosis of ASD seems untenable and wasteful, the use of many measurements operating in conjunction has gained traction. Dysregulation of amino acid metabolism was identified by comparing plasma metabolites from 516 children with ASD with a control group of 164 typical development children recruited into CAMP (Smith et al., 2019). Though a simple

analysis of the mean concentrations of free plasma amines did not reveal meaningful differences between the ASD and healthy populations of children, the researchers reported that a combination of glutamine, glycine, and ornithine amino acid dysregulation 'metabotypes' could be used to identify a dysregulation in amino acid/branch-chained amino acid metabolism that is present in 16.7% of the CAMP ASD subjects and is detectable with a specificity of 96.3% and a PPV of 93.5%, seemingly providing the grounds for metabolic testing (Smith et al., 2019).

Arizona State University's Comprehensive Nutritional and Dietary Intervention Study, a 12-month nutritional and dietary intervention study, compared plasma amino acid concentrations between ASD and typically developed individuals. The study included 64 study participants on the autism spectrum and 49 acted as age- and gender-matched typical development (TD) controls. In the clinical study, a total of 42 plasma amino acids and related metabolites were measured, including the nine essential and 11 non-essential amino acids as well as 22 secondary amino acids and amino acid metabolites (Vargason et al., 2018). However, even given the comprehensiveness of the study, at most could be said was that the results "indicate possibly elevated concentrations of glutamate, hydroxyproline, and serine in the plasma of individuals with ASD" but ultimately that "clear discrimination of the cohorts [ASD and TD cohorts] was not possible using these data," suggesting that the measurements themselves are less significant than previously anticipated (Vargason et al., 2018). However, a 2011 study in Arizona analyzed concentrations of 41 amino acids and amino acid metabolites in the plasma of 55 children with ASD and 44 TD children and detected significantly elevated glutamate and significantly decreased tryptophan in the ASD cohort (Adams et al., 2011). Similarly, a 2016 Chinese study reported that glutamate in the plasma of 51 children with ASD and 51 controls were significantly higher in the children with ASD consistent with the Arizona study (Cai et al., 2016). Therefore, aside from a singular common amino acid, the findings contradict other studies focusing on plasma amino acid measurements in ASD individuals that exalt and exhort it for its purported potential.

LaBianca et al. (2021) conducted a study on the relation between copy number variants (CNVs) and polygenic risk scores (PRS) on the extent of care needed for families with histories of autism and/or ADHD. They estimated that among a sample of 39 families, the overall variance explained by "known, rare, CNVs and SCZ [schizophrenia] PRS from common SNP [single nucleotide polymorphisms] to be 10% in comorbid ASD and ADHD." There was also a greater burden of both rare CNVs and SCZ PRS among adult ASD and/or ADHD patients with sustained needs of specialist care than unaffected relatives and any other relatives with mental health disorder. Although the study had a small sample size, having this application of CNVs and PRS can eventually predict care and benefit other families with ASD and/or ADHD, as well as assist in clinical decision making. Furthermore, CNV can be connected to autistic phenotypes. In a study by Chawner et al. (2021), they found that based on clinical cut-offs, four different genetic variant groups had differences in autism severity, IQ, and autism subdomain profiles, with a "substantial variability in phenotypic outcome within individual genetic variant groups" (74%-97%), with low variability between groups (1%-21% based on trait). For carriers, 54% with one of four CNVs that did not meet "full autism diagnostic criteria" still had "elevated levels of autistic traits." Collectively, these studies demonstrate that not only can CNVs render predictive value in treatment planning, but also understanding of presentation.

Genetic testing also yields pathogenic benefit. In a retrospective chart review by Harris et al. (2020) on 500 toddlers diagnosed with ASD per the *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition*, 59.8% completed genetic testing, with 12.0% yielding pathogenic findings from CMA and fragile X testing. The most common CNVs in this sample were "deletions or duplications on 15q (n=10) and 22q (n=2). Among subjects with fragile X findings, there were 3 full mutations, 3 pre mutations, 2 intermediate or "grey zone" mutations, and 1 patient with mosaicism." These pathogenic findings also

impacted medical recommendations 72.2% of those patients, showing how understanding pathogenesis in the setting of ASD can extend into not only identifying potential pathology but also clinical care once again. Bruno et al. (2021) used WES to investigate parent-offspring trios. The study included 60 trios, each of which included a patient (diagnosed with ASD or an ID-related phenotype) and their parents. The authors found eight pathogenic variants already known to be associated with ASD and ID (*SYNGAP1, SMAD6, PACS1, SHANK3, KMT2A, KCNQ2, ACTB,* and *POGZ*). The authors also found four novel candidate ASD/ID genes with de novo disruptive variants (*MBP, PCDHA1, PCDH15, PDPR).* The authors conclude that "these unknown rare variants, alone or in combination with each other, contributed to the phenotype." Further, the authors conclude that "data confirm the efficacy of WES in detecting pathogenic variants in known and novel ID/ASD genes" (Bruno et al., 2021).

Harrington et al. (2024) studied the ordering habits of providers to assess the diagnostic utility of genetic testing for ASD. The authors included data from a "large clinical laboratory" that was collected between 2017 and 2022. The authors found that females were 1.4 times more likely than males to receive a genetic diagnosis of ASD (95% CI:1.2-1.7). Overall, "exome had the highest diagnostic yield (24.5%), followed by NDD panel (6.4%), CMA (6.2%), and Fragile X testing (0.4%)." The authors concluded that "ASD testing should include exome, CMA, and other clinically indicated tests, as first-tier tests, with the consideration of panel testing, in cases where exome sequencing is not an option" (Harrington et al., 2024).

Guidelines and Recommendations

American Academy of Pediatrics (AAP)

In 2020, the American Academy of Pediatrics released extensive guidelines pertaining to the identification, evaluation, and management of children with ASD. The AAP notes, "The reported prevalence of children with ASD has increased over time… This increase may be attributable to several factors, including broadening in the diagnostic criteria with ongoing revisions of the Diagnostic and Statistical Manual of Mental Disorders (DSM), the more inclusive definition of pervasive developmental disorder with the adoption of the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) in 1994, increased public awareness of the disorder and its symptoms, recommendations for universal screening for ASD, and increased availability of early intervention and school-based services for children with ASD." The AAP goes on to explicitly recommend "screening all children for symptoms of ASD through a combination of developmental surveillance at all visits and standardized autism-specific screening tests at 18 and 24 months of age in their primary care visits because children with ASD can be identified as toddlers, and early intervention can and does influence outcomes. This autism-specific screening complements the recommended general developmental screening at 9, 18, and 30 months of age." They also recommend the use of the "Learn the Signs. Act Early" parent resources developed by the Centers for Disease Control and Prevention (CDC). Screening varies by age group, and screening results are not diagnostic. The results are to aid the primary care provider in identifying children who may require additional evaluation. For children younger than 18 months, the M-CHAT is the most studied tool, and the AAP notes that parent-administered questionnaires, such as the Communication and Symbolic Behavior Scales Development Profile and the Infant and Toddler Checklist, have been used to screen children as young as 12-months old. For children 18- to 30-months old, the most used screening tool is either the M-CHAT or M-CHAT-R/F (Modified Checklist for Autism in Toddlers, Revised with Follow-Up Questions). For children older than 30 months, "there are no validated screening tools available for use in pediatric practice, nor are there current recommendations by the AAP for universal screening for ASD in that age group" (Hyman et al., 2020).

Once a child has been determined to be at risk for ASD, the child should see a specialist, such as a neurodevelopmental or developmental/behavioral pediatrician, psychologist, neurologist, or a psychiatrist for a diagnostic evaluation. "At this time, there are no laboratory tests that can be used to make a diagnosis of ASD, so careful review of the child's behavioral history and direct observation of symptoms are necessary… Formal assessment of language, cognitive, and adaptive abilities and sensory status is an important component of the diagnostic process" (Hyman et al., 2020). The specialist may use questionnaire tools, such as the SCQ or Social Responsiveness Scale (SRS), or behavioral assessments, such as the Diagnostic Interview for Social and Communication Disorders (DISCO) or Child Behavior Checklist.

Concerning genetic testing, the AAP states that "[g]enetic evaluation should be recommended and offered to all families as part of the etiologic workup," and they provide a stepwise general approach as a practical guideline.

1. "Consider referral for pediatric genetics evaluation
2. Comprehensive history (including 3-generation family history with emphasis on individuals with ASD and other developmental, behavioral and/or psychiatric, and neurologic diagnoses)
    a. Physical examination (including dysmorphology, growth parameters [including head circumference], and skin examination)
        i. If syndrome diagnosis or metabolic disorder is suspected, go back to step 1 (genetics and/or metabolism referral) and/or order the appropriate targeted testing)
        ii. Otherwise, proceed to step 3
3. Laboratory studies
    a. Discuss and offer CMA analysis
    b. Discuss and offer fragile X analysis; if family history is suggestive of sex-linked intellectual disabilities, refer to genetics for additional testing
    c. If a patient is a girl, consider evaluation for Rett syndrome, *MECP2* testing
    d. If these studies do not reveal the etiology, proceed to step 4
4. Consider referral to genetics, workup might include WES" (Hyman et al., 2020).

Regarding the use of potential biomarkers for ASD, AAP states, "Although some studies have attempted to differentiate people with and without ASD on the basis of differences in laboratory profiles of platelet serotonin, plasma melatonin, urine melatonin sulfate, redo status, placental trophoblast inclusions, and immune function, currently no diagnostic laboratory tests have been approved for ASD. To date, none of these potential biomarkers under study has sufficient evidence to be recommended" (Hyman et al., 2020). AAP also notes, "The yield of routine metabolic testing for children with ASD is low and not recommended for regular use." However, they do note that there are uncommon metabolic disorders that may "rarely" be associated with ASD that may require necessary workup. These can include metabolic disorders involving amino acids, carnitine, folate, and cholesterol, for example (Hyman et al., 2020).

U.S. Preventive Services Task Force (USPSTF)

The USPSTF, in 2016, concluded "that the current evidence is insufficient to assess the balance of benefits and harms of screening for ASD in young children for whom no concerns of ASD have been raised by their parents or a clinician" (Siu et al., 2016). Within this evaluation, the USPSTF did not address

either genetic testing or the potential use of biomarkers for screening ASD. This guideline is currently being updated as of June 4, 2021.

The International Standard Cytogenomic Array (ISCA) Consortium

In 2010, the ISCA released a consensus statement that chromosomal microarray is a first-tier diagnostic test for individuals with developmental disabilities and delays, including individuals with ASD. The ISCA "strongly supports the use of CMA in place of G-banded karyotyping as the first-tier cytogenetic diagnostic test for patients with DD/ID, ASD, or MCA. G-banded karyotype analysis should be reserved for patients with obvious chromosomal syndromes (e.g. Down syndrome), a family history of chromosomal rearrangement, or a history of multiple miscarriages" (Miller et al., 2010).

American Academy of Child and Adolescent Psychiatry (AACAP)

Within the 2014 AACAP guidelines, they recommend that "clinicians should coordinate an appropriate multidisciplinary assessment of children with ASD… All children with ASD should have a medical assessment, which typically includes physical examination, a hearing screen, a Wood's lamp examination for signs of tuberous sclerosis, and genetic testing, which may include G-banded karyotype, fragile X testing, or chromosomal microarray" (Volkmar et al., 2014).

Centers for Disease Control and Prevention (CDC)

The CDC website for recommendations and guidelines for ASD supports the guidelines of the AAP (CDC, 2024a).

American College of Medical Genetics and Genomics (ACMG)

Within the 2013 ACMG guidelines, they recommend that a genetic consultation be offered to all individuals with ASD as well as their families. They also recommend the use of a tiered genetic diagnostic evaluation, consisting of the following:

- "First tier
  - Three-generation family history with pedigree analysis
  - Initial evaluation to identify known syndromes or associated conditions
    - Examination with special attention to dysmorphic features
    - If specific syndromic diagnosis is suspected, proceed with targeted testing
    - If appropriate clinical indicators present, perform metabolic and/or mitochondrial testing (alternatively, consider a referral to a metabolic specialist)
  - Chromosomal microarray: oligonucleotide array-comparative genomic hybridization or single-nucleotide polymorphism array
  - DNA testing for fragile X (to be performed routinely for male patients only)[a]
- Second tier
  - *MECP2* sequencing to be performed for all females with ASDs
  - *MECP2* duplication testing in males, if phenotype is suggestive
  - *PTEN* testing only if the head circumference is 2.5 SD above the mean
  - Brain magnetic resonance imagining only in the presence of specific indicators (e.g., microcephaly, regression, seizures, and history of stupor/coma)

  [a]DNA testing for fragile X in females if indicators present (e.g., family history and phenotype)" (Schaefer & Mendelsohn, 2013).

National Institute for Health and Care Excellence (NICE)

Concerning genetic testing in the NICE guidelines, they state, "Do not routinely perform any medical investigations as part of an autism diagnostic assessment, but consider the following in individual circumstances and based on physical examination, clinical judgment and the child or young person's profile: genetic tests, as recommended by your regional genetics centre, if there are specific dysmorphic features, congenital anomalies and/or evidence of a learning (intellectual) disability…." (NICE, 2017, 2021).

Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
81470	X-linked intellectual disability (XLID) (eg, syndromic and non-syndromic XLID); genomic sequence analysis panel, must include sequencing of at least 60 genes, including ARX, ATRX, CDKL5, FGD1, FMR1, HUWE1, IL1RAPL, KDM5C, L1CAM, MECP2, MED12, MID1, OCRL, RPS6KA3, and SLC16A2
81471	X-linked intellectual disability (XLID) (eg, syndromic and non-syndromic XLID); duplication/deletion gene analysis, must include analysis of at least 60 genes, including ARX, ATRX, CDKL5, FGD1, FMR1, HUWE1, IL1RAPL, KDM5C, L1CAM, MECP2, MED12, MID1, OCRL, RPS6KA3, and SLC16A2
0063U	Neurology (autism), 32 amines by LC-MS/MS, using plasma, algorithm reported as metabolic signature associated with autism spectrum disorder Proprietary test: NPDX ASD ADM Panel I Lab/Manufacturer: Stemina Biomarker Discovery, Inc
0170U	Neurology (autism spectrum disorder [ASD]), RNA, next-generation sequencing, saliva, algorithmic analysis, and results reported as predictive probability of ASD diagnosis Proprietary test: Clarifi™ Lab/Manufacturer: Quadrant Biosciences, Inc
0263U	Neurology (autism spectrum disorder [ASD]), quantitative measurements of 16 central carbon metabolites (i.e., α-ketoglutarate, alanine, lactate, phenylalanine, pyruvate, succinate, carnitine, citrate, fumarate, hypoxanthine, inosine, malate, S-sulfocysteine, taurine, urate, and xanthine), liquid chromatography tandem mass spectrometry (LC-MS/MS), plasma, algorithmic analysis

	with result reported as negative or positive (with metabolic subtypes of ASD) Proprietary test: NPDX ASD and Central Carbon Energy Metabolism Lab/Manufacturer: Stemina Biomarker Discovery, Inc
0322U	Neurology (autism spectrum disorder [ASD]), quantitative measurements of 14 acyl carnitines and microbiome-derived metabolites, liquid chromatography with tandem mass spectrometry (LC-MS/MS), plasma, results reported as negative or positive for risk of metabolic subtypes associated with ASD Proprietary test: NPDX ASD Test Panel III Lab/Manufacturer: Stemina Biomarker Discovery d/b/a NeuroPointDX

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

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Revision History

Revision Date	Summary of Changes
12/04/2024	Reviewed and Updated: Updated the background, guidelines and recommendations, and evidence-based scientific references. Literature review did not necessitate any modifications to coverage criteria. The following edits were made for clarity: Title changed from "Testing for Autism Spectrum Disorder and Developmental Delay" to "Testing for Developmental Delay" CC1 edited for clarity, now reads: "1) For individuals less than 18 years of age who have had a physical examination suggestive of syndromic developmental delay or developmental delay due to a metabolic disorder (e.g., dysmorphology, growth parameters [including head circumference], skin examination), targeted genetic testing MEETS COVERAGE CRITERIA." CC2 edited to reflect that all other tests other than types addressed in other policies (CMA, WES, WGS, FXS, RTT) are not appropriate for ASD or non-syndromic developmental delay, moves below disclaimer about testing not meeting criteria due to a lack of scientific support. Now reads: "2) For the diagnosis of autism spectrum disorder (ASD) or non-syndromic developmental delay, all other testing outside of chromosomal microarray, whole exome sequencing, or whole genome sequencing or genetic testing for fragile X syndrome or Rett syndrome DOES NOT MEET COVERAGE CRITERIA." Note was updated to reflect changes to Avalon's definition of a genetic panel within R2162. Now reads: "Note: For two or more gene tests being run on the same platform, please refer to AHS-R2162-Reimbursement Policy."

Testing for Diagnosis of Active or Latent Tuberculosis

Policy Number: AHS – G2063 – Testing for Diagnosis of Active or Latent Tuberculosis	Prior Policy Name and Number, as applicable: • AHS – G2063 – Gamma Interferon Blood Test for Diagnosis of Latent Tuberculosis
Initial Presentation Date: 09/18/2015 Revision Date: February 1, 2025	

Policy Description

Infection by *Mycobacterium tuberculosis* (Mtb) results in a wide range of clinical presentations dependent upon the site of infection from classic signs and symptoms of pulmonary disease (cough greater than two to three weeks' duration, lymphadenopathy, fevers, night sweats, weight loss) to silent infection with a complete absence of signs or symptoms (Lewinsohn et al., 2017).

Culture of Mtb is the gold standard for diagnosis as it is the most sensitive and provides an isolate for drug susceptibility testing and species identification (Bernardo, 2024). Nucleic acid amplification tests (NAAT) use polymerase chain reactions (PCR) to enable sensitive detection and identification of low-density infections (Pai et al., 2004). Interferon-gamma release assays (IGRAs) are blood tests of cell-mediated immune response which measure T cell release of interferon (IFN)-gamma following stimulation by specific antigens such as *Mycobacterium tuberculosis* antigens (Lewinsohn et al.,

2017; Menzies, 2024) used to detect a cellular immune response to *M. tuberculosis* which would indicate latent tuberculosis infection (LTBI) (Pai et al., 2014).

Related Policies

Policy Number	Policy Title
N/A	

Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) To diagnose or screen for latent tuberculosis (TB) infection, an interferon gamma release assay (IGRA) **MEETS COVERAGE CRITERIA** in:
   a) Individuals who are at risk for infection with *Mycobacterium tuberculosis* (Mtb).
   b) Individuals who are unlikely to be infected with Mtb when screening is obliged by law.
2) For all suspected TB infections, the following tests **MEET COVERAGE CRITERIA**:
   a) Acid fast bacilli (AFB) smear/stain.
   b) Culture and culture-based drug susceptibility testing of *Mycobacteria* spp.
3) Direct probe or amplified probe nucleic acid-based testing, including PCR, **MEETS COVERAGE CRITERIA** for **any** of the following:
   a) *Mycobacteria* spp.
   b) *M. tuberculosis*.
   c) *M. avium intracellulare*.
4) For individuals whose sputum is AFB smear positive or Hologic Amplified MTD positive, molecular-based drug susceptibility testing **MEETS COVERAGE CRITERIA** when **one** of the following criteria is met:
   a) The individual has been treated for TB in the past.
   b) The individual was born in or has lived for at least 1 year in a foreign country with at least a moderate TB incidence (≥20 per 100, 000) or a high primary multi-drug resistant (MDR)-TB prevalence (≥2%).
   c) The individual is a contact of an individual with MDR-TB.

d) The individual is HIV infected.

5) Repeat drug susceptibility testing **MEETS COVERAGE CRITERIA** in **any** of the following situations:
   a) For individuals whose sputum cultures remain positive after 3 months of treatment.
   b) When there is bacteriological reversion from negative to positive.

6) For individuals with pleural effusion, pericardial effusion, or ascites and suspected TB infection, cell counts, protein, glucose, and lactate dehydrogenase (LDH) concentrations of cerebrospinal, pleural, peritoneal, pericardial, and other fluids **MEETS COVERAGE CRITERIA**.

7) In HIV-infected individuals with CD4 cell counts ≤100 cells/microL who have signs and symptoms of tuberculosis, urine-based detection of mycobacterial cell wall glycolipid lipoarabinomannan (LAM) **MEETS COVERAGE CRITERIA**.

8) For individuals with active tuberculosis, IGRA **DOES NOT MEET COVERAGE CRITERIA**.

9) Simultaneous ordering of any combination of direct probe, amplified probe, and/or quantification for the same organism in a single encounter **DOES NOT MEET COVERAGE CRITERIA**.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

10) Quantitative nucleic acid testing for *Mycobacterium* spp, *M. tuberculosis*, and *M. avium intracellulare* **DOES NOT MEET COVERAGE CRITERIA**.

11) Whole genome sequencing of *Mycobacterium* spp. for the detection of drug resistance **DOES NOT MEET COVERAGE CRITERIA**.

12) Genotyping of *Mycobacterium* spp. **DOES NOT MEET COVERAGE CRITERIA**.

13) Testing of adenosine deaminase (ADA) and interferon-gamma (IFN- γ) levels in cerebrospinal, pleural, peritoneal, pericardial, and other fluids for the diagnosis of extrapulmonary TB **DOES NOT MEET COVERAGE CRITERIA**.

14) Testing of serum protein biomarkers or panels of biomarkers for the detection and diagnosis of TB **DOES NOT MEET COVERAGE CRITERIA**.

Table of Terminology

Term	Definition
ADA	Adenosine deaminase
AFB	Acid fast bacilli
ASM	American Society of Microbiology
ATS	American Thoracic Society
BCG	Bacillus Calmette-Guérin
CCs	Critical concentrations
CD4	Cluster of differentiation 4
CDC	Centers for Disease Control and Prevention
CLIA ’88	Clinical Laboratory Improvement Amendments of 1988
CMS	Centers for Medicare and Medicaid Services
CSF	Cerebrospinal fluid
DOR	Diagnostic odds ratio
DR-TB	Drug resistant tuberculosis
DST	Drug susceptibility testing
DSTs	Drug susceptibility tests
ECDC	European Centre for Disease Prevention and Control
ELISA	Enzyme-linked immunosorbent assay
ELISPOT	Enzyme-linked immunospot
ERS	European Respiratory Society
FDA	Food and Drug Administration
HIV	Human immunodeficiency virus
IDSA	Infectious Diseases Society of America
IFN	Interferon
IFN- γ	Interferon gamma
IGRA	Interferon gamma release assay
LAM	Lipoarabinomannan
LD/LDH	Lactate dehydrogenase
LDTs	Laboratory-developed tests
LF-LAM	Lipoarabinomannan assay
LPAs	Line probe assays
LTBI	Latent tuberculosis infection
MDR	Multi-drug resistant

MIC	Minimum inhibitory concentration
MMR	Measles-mumps-rubella
Mtb	*Mycobacterium tuberculosis*
MTBC	*Mycobacterium tuberculosis* complex
MTD	*Mycobacterium tuberculosis* direct
NAA	Nucleic acid amplification
NAAT	Nucleic acid amplification tests/techniques
NICE	National Institute for Health and Care Excellence
NIH	National Institute of Health
NLR	Negative likelihood ratio
NPF	National Psoriasis Foundation
NPV	Negative predictive value
NSTC	National Society of Tuberculosis Clinicians
NTCA	National Tuberculosis Controllers Association
NTM	Non-Tuberculosis Mycobacterium Species
OR	Odds ratio
PCR	Polymerase chain reactions
pDST	Phenotypic drug susceptibility testing
PLR	Positive likelihood ratio
PPV	Positive predictive value
QFT-G/QFT-GT	Quantiferon- Tuberculosis Gold
QFT-GIT	Quantiferon- Tuberculosis Gold In-Tube
RBS	Rapid biosensor
RIF	Rifampicin
RR-TB	Rifampicin-resistant tuberculosis
SL-LPA	Second-line line probe assays
TB	Tuberculosis
TBNET	Tuberculosis Network European Trials Group
TNF	Tumor necrosis factor
TNF- α	tumor necrosis factor–α
TNFi	Tumor necrosis alpha inhibitor
TST	Tuberculin skin tests
WGS	Whole-genome sequencing
WHO	World Health Organization

Scientific Background

Tuberculosis (TB) continues to be a major public health threat globally, causing an estimated 10.0 million new cases and 1.2 million deaths from TB among HIV-negative individuals and 208,000 deaths among HI-positive people in 2019 (WHO, 2020), with the emergence of multidrug resistant strains only adding to the threat (Dheda et al., 2014). The lungs are the primary site of infection by Mtb and subsequent TB disease. Onset of symptoms is usually gradual with a persistent cough being most frequently reported (95%) followed by the typical symptoms of fever (75%), night sweats (45%) and weight loss (55%) (Heemskerk et al., 2015). Clinical manifestations include primary TB, reactivation TB, laryngeal TB, endobronchial TB, lower lung field TB infection, and tuberculoma (Bernardo, 2024). Extrapulmonary infection represents approximately 20% of cases of active TB with an additional 7% having concurrent pulmonary and extrapulmonary infections (Peto et al., 2009).

In most individuals, initial *Mycobacterium tuberculosis* infection is eliminated, or contained by host defenses, while infection remains latent (Barry et al., 2009; Dheda et al., 2010). Persons with latent TB infection (LTBI) are considered to be asymptomatic and not infectious; however, latent Mtb bacilli may remain viable and reactivate to cause active, contagious infection. Identification and treatment of LTBI are important TB control strategies, especially in settings with a low TB incidence, where reactivation of LTBI often accounts for the majority of nonimported TB disease (ATS, 2000; Landry & Menzies, 2008; Pai et al., 2014).

Latent TB Testing (LTBI)

The goal of testing for LTBI is to identify individuals who are at increased risk for the development of tuberculosis (TB) and therefore who would benefit from treatment of latent TB infection. Only those who would benefit from treatment should be tested so a decision to test presupposes a decision to treat if the test is positive (Menzies, 2024).

Proprietary Testing

The Bactec MGIT 960 System was approved by the FDA in 1998 for the detection of mycobacteria growth from clinical specimens (except blood). In 1994 the FDA approved the Ge-Probe Amplified Mycobacterium Tuberculosis Direct Test as a Nucleic acid-based in vitro diagnostic devices for the detection of *Mycobacterium tuberculosis* complex in respiratory specimens. These devices are non-multiplexed and intended to be used as an aid in the diagnosis of pulmonary tuberculosis when used in conjunction with clinical and other laboratory findings (Lewinsohn et al., 2017).

In 2015 the FDA approved the Xpert® MTB/RIF Assay, performed on the GeneXpert® Instrument Systems, as a qualitative, nested real-time polymerase chain reaction (PCR) in vitro diagnostic test for the detection of *Mycobacterium tuberculosis* complex DNA in raw sputum or concentrated sputum sediment prepared from induced or expectorated sputum. In specimens where *Mycobacterium tuberculosis* complex (MTB-complex) is detected, the Xpert MTB/RIF Assay also detects the rifampin-resistance associated mutations of the *rpoB* gene (Lewinsohn et al., 2017).

The QuantiFERON-TB® assay (CSL Biosciences, Australia) for detection of gamma interferon production is a blood test that has been used in humans in Australia. In November 2001, this test received approval from the U.S. Food and Drug Administration (FDA) in the United States for the following indication: "The QuantiFERON-TB test is intended as an aid in the detection of latent Mycobacterium tuberculosis infection" (FDA, 2001).

In December of 2004, QuantiFERON-TB® GOLD received FDA approval for the detection of latent TB. This test differs from the first-generation test in that instead of using PPD as the stimulus for interferon production, two antigens, ESAT-6 and CFP-10, are used. These antigens are present in mycobacterium tuberculosis but are not present in those exposed to BCG or non-tuberculous mycobacteria (Lewinsohn et al., 2017).

The QFT-GIT measures IFN-γ plasma concentration using an enzyme-linked immunosorbent assay (ELISA), has been approved by the US Food and Drug Administration (FDA) and has replaced the QuantiFERON-TB Gold (QFT-G) test (Lewinsohn et al., 2017).

The T-SPOT assay enumerates T cells releasing IFN-γ using an enzyme-linked immunospot (ELISPOT) assay. The T-SPOT.TB assay is currently available in Europe, Canada, and has been approved for use in the United States with revised criteria for test interpretation (Lewinsohn et al., 2017)

Analytical Validity

Mycobacterial infection results in a predominantly cell-mediated immune response (Daniel, 1980). Skin testing (TST) has long been a convenient, cost-effective method for assessing cell-mediated immune responses to a variety of antigens and has been the "gold standard" for diagnostic screening for *Mycobacterium tuberculosis* infections. However, multiple factors challenge the accuracy of the skin test, including skill requirements for and variability in placement and reading, cross-reactivity, and underlying illness or immunosuppression (Daniel, 1980). The sensitivity of the TST is approximately 71%–82% (Francis et al., 1978; Katial et al., 2001; Lewinsohn et al., 2017).

The cell-mediated immune response to *M. tuberculosis* involves production of gamma interferon (IFN-γ) (Fenton et al., 1997). Interferon-gamma release assays (IGRAs), which are in-vitro culture assays measuring IFN-γ production in response to tuberculin antigen stimulation, have been developed as diagnostic screening tests (Katial et al., 2001; Lein & Von Reyn, 1997) IGRAs have specificity >95% for diagnosis of latent TB infection and a sensitivity of 80-90% (Menzies et al., 2007; Pai et al., 2014). The two commercially available IGRAs are the QuantiFERON-TB Gold In-Tube (QFT-GIT) assay and T-SPOT.TB assay. Both assays are FDA-approved and available worldwide. These tests are not used to diagnose an active infection (as active infections are microbiologic diagnoses), but they still provide use as a confirmatory test for the TST (Menzies, 2024).

Clinical Utility and Validity

LTBI Testing

Diel et al. (2012) performed a meta-analysis investigating the "positive and the negative predictive value (PPV and NPV, respectively) from a test-determined LTBI state for progression to active TB of interferon-γ release assays (IGRAs) and the tuberculin skin test (TST)." The authors found that the "pooled PPV for progression for all studies using commercial IGRAs was 2.7% compared with 1.5% for the TST." PPV was found to increase to 6.8% and 2.4% respectively when only high-risk groups were included. The authors concluded that "Commercial IGRAs have a higher PPV and NPV for progression to active TB compared with those of the TST"(Diel et al., 2012).

Ruan et al. (2016) further assessed the "diagnostic value of interferon-γ release assays (IGRAs) for latent tuberculosis infection (LTBI) in patients with rheumatic disease before receiving biologic agents." 11 studies (n = 1940) were included. The authors found that "compared with the tuberculin skin test (TST), the pooled agreements in QFT-G/GIT and T-SPOT.TB were 72% and 75%, respectively. BCG vaccination was positively correlated with positive rates of TST (pooled odds ratio [OR] 1.64). Compared with TST, IGRAs were better associated with the presentence of one or more tuberculosis (TB) risk factors." The authors concluded that "in rheumatic patients with previous BCG vaccination or currently on steroid therapy, IGRAs would be the better choice to identify LTBI by decreasing the false-positivity and false-negativity rate compared with conventional TST" (Ruan et al., 2016).

Auguste et al. (2017) compared IGRA and TST for identifying latent tuberculosis infection that progresses to active tuberculosis. A total of 17 studies were included. However, no significant differences were observed, and the authors concluded that "prospective studies comparing IGRA testing against TST on the progression from LTBI to TB were sparse, and these results should be interpreted with caution due to

uncertainty, risk of bias, and unexplained heterogeneity. Population-based studies with adequate sample size and follow-up are required to adequately compare the performance of IGRA with TST in people at high risk of TB" (Auguste et al., 2017).

Nasiri et al. (2019) performed a meta-analysis focusing on the diagnostic accuracy of IGRA and TST for LTBI in transplant patients. A total of 16 articles were included, and the results are as follows: "pooled sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (PLR), negative likelihood ratio (NLR) and diagnostic odds ratio (DOR) for TST were 46%, 86%, 46.3%, 88.7%, 3.3, 0.63, and 5 respectively. For QFT-G (an IGRA), the pooled sensitivity, specificity, PPV, NPV, PLR, NLR, and DOR were 58%, 89%, 72.7%, 80.6%, 5.3, 0.47, and 11, respectively. Likewise, for T-SPOT.TB (another IGRA), the pooled sensitivity, specificity, PPV, NPV, PLR, NLR, and DOR were 55%, 92%, 60.4%, 90.2%, 6.7, 0.52, and 16, respectively." The authors concluded that "IGRAs were more sensitive and specific than the TST with regard to the diagnosis of LTBI in the transplant candidates. They have added value and can be complementary to TST" (Nasiri et al., 2019).

Khanna et al. (2021) performed a retrospective review of QuantiFERON TB test (QFT) results to evaluate the utility of serial LTBI screening in patients taking biologics and to identify risk factors in patients. They found that "Repeat LTBI testing in patients taking biologics revealed a low rate of conversion (1.17%)," and concluded that their "results suggest clinical utility and cost-effectiveness of repeat LTBI screening in patients on biologics may be more valuable if not performed routinely, but driven by a focused review of TB exposure risk factors in each patient" (Khanna et al., 2021).

In a prospective observational study conducted in a tertiary care center in India, Neema et al. (2021) found that "Treatment of LTBI prevents up to 60–70% patients from developing active tuberculosis; however, a patient may develop active tuberculosis despite prophylaxis especially with TNF inhibitors and patient should be followed up regularly." As such, "A thorough search for active tuberculosis should be performed. Timely detection of LTBI helps in the prevention of development of active tuberculosis in the patients on immunosuppressive treatment." However, it should be noted that the target study population for this study is the Indian population, which the authors acknowledged has a high prevalence of tuberculosis and latent tuberculosis infection already, and so it is unclear if the value may not be extrapolated to other populations.

Ren et al. (2024) studied the sensitivity of interferon-γ release assays (IGRAs) in identifying MTB-infected individuals. The study included 302 individuals, assigned into the following groups: healthy control, LTBI, IGRA-positive TB, and IGRA-negative TB. The Luminex xMAP assay was used to measure MTB antigen-specific blook plasma

chemokine concentrations. “Levels of CXCL9, CXCL10, IL-2, and CCL8 biomarkers were predictive for active TB.” The CXCL9-based enzyme-linked immunosorbent assay sensitivity rate was 95.9% and the specificity rate as 100%. CXCL9 and CXCL9-CXCL10 assays had statistically similar area under the curve values, “thus demonstrating that combined analysis of CXCL10 and CXCL9 levels did not improve active TB diagnostic performance.” The authors concluded that “The MTB antigen stimulation-based CXCL9 assay may compensate for low IGRA diagnostic accuracy when used to diagnose IGRA-negative active TB cases and thus is an accurate and sensitive alternative to IGRAs for detecting MTB infection” (Ren et al., 2024).

Active TB Testing

The diagnosis of TB disease should be suspected in patients with relevant clinical manifestations and exposure history (Lewinsohn et al., 2017). Laboratory testing is an integral part of the rapid and accurate diagnosis of TB to facilitate timely initiation of treatment.

Microbiologic testing is used to evaluate an active TB infection. These tests may include the acid-fast bacilli smear (AFB), the mycobacterial culture, and molecular testing. Smears are the fastest and cheapest diagnostic tool, cultures are the most sensitive, and molecular testing is used for assessing drug resistance (Bernardo, 2024).

The detection of acid-fast bacilli (AFB) on microscopic examination of stained sputum smears is the most rapid and inexpensive technique (Bernardo, 2024); however, it is limited by its lack of sensitivity in certain situations, such as extrapulmonary infection or coinfection with HIV (Pai et al., 2016). The mycobacteria retain the stain in a mineral-acid or acid-alcohol solution, and microscopy identifies these strains. LED microscopy has seen more use recently than the traditional light microscopy (Bernardo, 2024).

Rapid and accurate diagnosis is critical for timely initiation of TB treatment (Pai et al., 2016). Although sensitive, culture can take over two weeks to return results (Lewinsohn et al., 2017). Three specimens should be examined to assure a sensitivity of approximately 70%. The first specimen has a sensitivity of approximately 53.8%, increasing by 11.1% with a second specimen, and another 2-5% with a third (Mase et al., 2007). A first morning specimen increases sensitivity by 12%, and concentrating specimens can increase sensitivity by 18% (Steingart, Ng, et al., 2006). Use of fluorescence microscopy also increases sensitivity 10% over conventional microscopy (Lewinsohn et al., 2017; Steingart, Henry, et al., 2006). The positive predictive value has been reported to be 97.9-100% (Gordin & Slutkin, 1990), but it is impacted by non-tuberculosis *Mycobacterium* species (NTM) (Yajko et al., 1994).

Nucleic acid amplification techniques (NAAT) have been developed for rapid diagnosis of TB. Two major tests are available, the Amplified Mycobacterium tuberculosis Direct (MTD) test and the Xpert MTB/RIF test. NAAT-based assays are more sensitive than smear, but less sensitive than culture, with a reported sensitivity of 96% and specificity of 99% (Greco et al., 2006; Lewinsohn et al., 2017). NAAT testing has >95% positive predictive value in the setting of AFB smear-positive specimens for distinguishing tuberculous from nontuberculous mycobacteria, and it can establish the presence of tuberculosis in 50 to 80% of AFB smear-negative specimens (Cheng et al., 2005). NAAT does not replace the roles of AFB smear and culture (Ling et al., 2008) in the diagnostic algorithm for tuberculosis and results must be interpreted in conjunction with AFB smear results while mycobacterial culture is pending (CDC, 2009; Lewinsohn et al., 2017).

Sequence-based assays provide the genetic identity of a particular mutation and, therefore, can predict drug resistance with greater accuracy than probe-based assays. The testing identifies genetic mutations associated with rifampin and isoniazid resistance as well as resistance to second-line drugs including fluoroquinolones and the injectables amikacin, kanamycin, and capreomycin. Molecular testing results are generally available within days and can be used to guide initial treatment decisions and inform design of prevention regimens for contacts (Bernardo, 2024; Taylor et al., 2005).

More proprietary tests exist for the assessment of TB. Rapid Biosensor (RBS) offers a breath test "TB Breathalyzer" for TB. The test proposes that it can detect actively infectious bacilli instead of relying on sputum (which some patients do not produce). The test estimates its limit of detection at 25-75 bacilli and notes that it can be used easily in rural communities. When a patient coughs in the collection tube, any TB bacilli will react with the biochemical formulation at the bottom of the tube, which is then detected by the diode laser in the reader unit (RBS, 2015).

The reference standard for diagnosis of any TB infection is isolation of *M. tuberculosis* (Pai et al., 2016). The isolate recovered should be identified according to the Clinical and Laboratory Standards Institute guidelines (Institute, 2018) and the American Society for Microbiology Manual of Clinical Microbiology (Lewinsohn et al., 2017; Woods et al., 2015), and all United States jurisdictions require submission of culture isolates identified as *M. tuberculosis* for confirmation of identification and drug susceptibility testing (Taylor et al., 2005). Positive cultures are also reported to public health authorities for oversight and case management (Bernardo, 2024).

Cruciani et al. (2004) performed a meta-analysis of 10 studies (1381 strains from 14745 clinical specimens) which found that both liquid and solid culture media methods are

highly specific (99%). Liquid culture methods are more sensitive (81.5-85.8%) and have a shorter time to detection (13.2-15.2 day) than solid media but are more prone to contamination (4-9%). Solid media has a sensitivity of 76% and averages 25.8 days for detection. The use of both culture methods increases the overall sensitivity to 87.7-89.7%.

Bourgi et al. (2017) "aimed to evaluate the reliability and projected impact of nucleic acid amplification (NAA) testing in patients with acid-fast bacilli (AFB) smear-positive respiratory samples." The authors identified a retrospective cohort of AFB smear-positive patients and evaluated the projected change in "duration of airborne isolation and unnecessary Mycobacterium tuberculosis (MTB) treatment with introducing NAA testing into clinical decision making for AFB smear-positive patients." A total of 130 patients were found to be AFB positive, of which 80 tested positive on NAA. 82 patients grew MTB on culture. NAA testing was found to have a sensitivity of 97.6% and specificity of 100%. Integrating NAA testing into clinical decision making led to shortened time in airborne isolation (6.0 ± 7.6 vs 23.1 ± 38.0) and 9.5 ± 11.32 fewer days of "unnecessary MTB treatment in patients with negative NAA test." The authors concluded, "Nucleic acid amplification testing provided a rapid and accurate test in the diagnosis of MTB while significantly reducing the duration of isolation and unnecessary medications in patients with negative NAA test" (Bourgi et al., 2017).

Urine testing for mycobacterial cell wall glycolipid (Shah et al., 2010) has been investigated as a point of care assay for diagnosis of TB in HIV infected patients (Nakiyingi et al., 2014). The test was 97.6% specific and 67.9% sensitive in patients with CD4<100. It is useful in addition to routine diagnostic tests for HIV-infected patients with signs and symptoms of TB and CD4 ≤100 cells/microL and for all HIV-infected patients who are seriously ill (Shah et al., 2016; WHO, 2015a). Gupta-Wright et al. (2018) evaluated the sputum Xpert MTB/RIF with or without urine lipoarabinomannan (LAM) testing. There was no difference in overall mortality over 2574 patients, but they found that urine Lam testing might benefit some high-risk subgroups (CD4 <100, severe anaemia, and patients with clinically suspected tuberculosis) (Gupta-Wright et al., 2018).

Adenosine deaminase (ADA) and interferon-gamma (IFN- γ) levels in cerebrospinal, pleural, peritoneal, and pericardial fluids have been studied in the diagnosis of extrapulmonary TB. In 2017, a joint review by the ATS, IDSA, and CDC found the sensitivity of ADA in these fluids to be 79% and the specificity to be 83% for TB. The sensitivity of IFN- γ in these fluids was 89% and the specificity was 97%. However, the authors remarked that neither the ADA level nor the IFN-γ level provide a definitive diagnosis of TB disease (Lewinsohn et al., 2017).

De Groote et al. (2017) developed a panel based on proteomic analysis. A total of 1470 serum samples were collected from patients "with symptoms and signs suggestive of active pulmonary TB that were systematically confirmed or ruled out for TB by culture and clinical follow-up." Six protein biomarkers were identified: "SYWC, kallistatin, complement C9, gelsolin, testican-2, and aldolase C," which performed well in a training set (area under curve = 0.92) to distinguish between TB and non-TB. It was also found to have 90% sensitivity and 80 % specificity. The authors concluded that their panel "warrants diagnostic development on a patient-near platform" (De Groote et al., 2017).

Heyckendorf et al. (2018) compared the utility of genotypic and phenotypic assays for evaluation of tuberculosis (TB) drug resistance. The authors used the results from the assays to develop treatment regimens for the 25 multi- and extensively drug-resistant tuberculosis patients in the study. Compared to phenotypic assay-developed regimens, whole genome sequencing (WGS) yielded a regimen of drugs at 93% agreement with the phenotypic assay's regimen. Further, the whole genome sequencing-derived regimen did not contain any drugs identified as resistant by the phenotypic assay. However, the authors commented that "MIC [minimum inhibitory concentration] testing revealed that pDST [phenotypic drug susceptibility testing] likely underestimated the true rate of resistance for key drugs (rifampin, levofloxacin, moxifloxacin, and kanamycin) because critical concentrations (CCs) were too high." Results derived from other genotypic assays (Xpert, line probe assays) had lower agreement with the phenotypic assay (49% and 63% respectively). The authors concluded that "WGS can be used to rule in resistance even in M/XDR strains with complex resistance patterns, but pDST for some drugs is still needed to confirm susceptibility and construct the final regimens. Some CCs for pDST need to be reexamined to avoid systematic false-susceptible results in low-level resistant isolates" (Heyckendorf et al., 2018).

Ustinova et al. (2019) investigated an assay's ability to identify and distinguish between nontuberculous mycobacteria (NTM) and *Mycobacterium tuberculosis* complex (MTBC) in culture and sputum. A total of 301 NTM cultures with mycobacteriosis were measured, and sputum samples were contributed by "104 patients with mycobacteriosis, 3627 patients with tuberculosis and 118 patients with other lung diseases." The authors results were as follows: "Specificity and sensitivity of the assay for MTBC was found to be 100% both in culture and sputum samples; for NTM, the specificity was 100% in culture and sputum, the sensitivity reached 100% in culture and 73.1% in sputum samples. Positive predictive value (PPV) and negative predictive value (NPV) of the assay for culture were both 100%, for clinical material 100% and 80.8%, respectively" (Ustinova et al., 2019).

Adams et al. (2019) compared the performances of the tuberculin skin test (TST) and two interferon-gamma-release assays (IGRAs). Five hundred and five health care workers (HCWs) in Cape Town, South Africa, were screened for latent tuberculosis infection (LTBI) using the three assays. The authors identified LTBI prevalence to be 81%. TST at a cut off of 10 mm had the highest sensitivity at 93% and the lowest specificity at 57%. The QFT-GIT IGRA sensitivity was 80% and specificity was 96%; the TSPOT.TB IGRA sensitivity was 74% and specificity was 96%. Positive predictive values for IGRAs was 90% and 96% for TST and the highest negative predictive value was 66%. However, a composite rule using both TST and QFT-GIT improved negative predictive value to 90%. The authors concluded that "in an endemic setting a positive TST or IGRA was highly predictive of LTBI, while a combination of TST and IGRA had high rule-out value (Adams et al., 2019).

Zürcher et al. (2019) evaluated the "mortality in patients with tuberculosis from high-burden countries, according to concordance or discordance of results from drug susceptibility testing done locally and in a reference laboratory." A total of 634 patients were included, 272 of which were HIV-positive. The authors identified 394 strains (62%) to be "pan-susceptible," 45 (7%) to be monoresistant, 163 (27%) to be multi-drug resistant, and 30 (5%) to be "extensively" resistant. The laboratory results were concordant for 513 (81%) patients and discordant for 121 (19%) patients, resulting in a 90.8% sensitivity and 84.3% specificity. The authors identified a 7.33 odds ratio of death for patients with discordant results, which potentially led to under-treatment. The authors concluded "inaccurate drug susceptibility testing by comparison with a reference standard leads to under-treatment of drug-resistant tuberculosis and increased mortality" (Zürcher et al., 2019).

Jain et al. (2021) conducted a cross-sectional study in India to "compare the performance of GeneXpert MTB/RIF (GXpert) assay with [the] composite reference standard in diagnosing cases of tubercular pleural effusion (TPE) and to evaluate the reliability of rifampicin resistance." In diagnosing TPE, the sensitivity of the assay was 16.6% among 158 study participants, with a specificity of 100%, diagnostic accuracy of 52.5%, positive predictive value of 100%, and negative predictive value of 47.5%. Because of these findings, the researchers concluded that this GXpert assay would need to be combined with "routine pleural fluid analysis" to accurately diagnose TPE in suspected patients (Jain et al., 2021).

Karthek et al. (2021) evaluated the usage of the same GeneXpert MTB/RIF assay in the context of spinal tuberculosis. In conducting a retrospective review from 136 patients that underwent spinal biopsy for spondylodiscitis, 86 final patients met the criteria for spinal tuberculosis (61.6% demonstrated Mtb positivity in tissue samples and 38.4% were positive through pus samples). From this data, the researchers found a 65.1%

sensitivity, 100% specificity, 100% PPV, and 56.5% NPV for this assay. It was also accurate in detecting drug resistance among patient specimen (Karthek et al., 2021).

Medina-Marino et al. (2024) conducted a randomized study in South Africa to assess the acceptability and feasibility of in-home TB testing of household contacts. The study included 84 households with at least one eligible symptomatic contact, for a total of 98 household contacts. The household contacts were all randomized, with 51 receiving in-home testing and 47 receiving standard-of-care. In-home testing included GeneXpert MTB/RIF molecular testing, and referrals for clinic-based treatment for positive cases. Standard-of-care testing included clinic-based sputum collection and testing. The median number of days between screening and receiving testing results was zero for the in-home testing group, and 16.5 for the standard-of-care testing group. The authors concluded that "in-home testing for TB was acceptable, feasible, and increased HHCs with a molecular test result" and that "in-home testing mitigates a major limitation of household contact investigations (dependency on clinic-based referral), revealing new strategies for enhancing early case detection" (Medina-Marino et al., 2024).

Guidelines and Recommendations

World Health Organization (WHO)

The WHO published recommendations for the diagnosis of TB, stating that:

- "Mycobacteria can be visually distinguished from other microorganisms by their thick lipid containing cell walls, which retain biochemical stains despite decolourization by acid-containing reagents (known as 'acid fastness'). Given that the examination of two sputum specimens is adequate to identify the majority (95-98%) of smear-positive TB patients, WHO's current policy on case-finding using microscopy recommends that in settings with appropriate external quality assessment and documented good-quality microscopy two specimens should be examined" (WHO, 2015b).
- "Direct Ziehl–Neelsen staining of sputum specimens and examination using light microscopy is suitable for use at all levels of laboratory, including peripheral laboratories at primary health-care centres or district hospitals. There is insufficient evidence that processed sputum specimens (for example, those that are concentrated or chemically treated) give better results than direct smear microscopy. Therefore, the use of such methods is not recommended" (WHO, 2015b).
- "Evidence shows that the diagnostic accuracy of LED microscopy is comparable to that of conventional fluorescence microscopy and it surpasses that of conventional Ziehl–Neelsen microscopy (by an average of 10%). Therefore, WHO

recommends replacing conventional fluorescence microscopy with LED microscopy, and that LED microscopy should be phased in as an alternative to conventional Ziehl–Neelsen light microscopy in all settings, prioritizing high-volume laboratories" (WHO, 2015b).

- "Mycobacteria can be cultured in specific solid or liquid media. Bacterial growth can be identified visually (that is, by identifying specific characteristics) or by automated detection of its metabolism. All positive mycobacterial cultures must be tested to confirm the identification of *M. tuberculosis* complex (MTBC)" (WHO, 2015b).
- "Differentiation of the members of the MTBC is necessary for the treatment of individual patients and for epidemiological purposes, especially in areas of the world where tuberculosis has reached epidemic proportions or wherever the transmission of M. bovis between animals or animal products and humans is a problem. In addition, it can be important to rapidly identify isolates of M. bovis bacillus Calmette-Guérin (BCG) recovered from immunocompromised patients. Differentiation of species with the MTBC can be achieved using either phenotypic26 and/ or genotypic methods" (WHO, 2015b).
- "The use of rapid immunochromatographic assays (or strip tests for speciation) to identify cultured isolates is recommended because they provide definitive identification of all members of the MTBC (including M. bovis) in 15 minutes" (WHO, 2015b).
- "WHO recommends that either TST or IGRA can be used to test for LTBI in high-income and upper middle-income countries with estimated TB incidence less than 100 per 100000 population" (WHO, 2015a).
- "It is strongly recommended that commercial serodiagnostic tests not be used for the diagnosis of pulmonary and extra-pulmonary TB. Currently available commercial serodiagnostic tests (also referred to as serological tests) provide inconsistent and imprecise findings. There is no evidence that existing commercial serological assays improve patient outcomes, and high proportions of false positive and false-negative results may have an adverse impact on the health of patients" (WHO, 2015b).
- "There is no consistent evidence that IGRAs are more sensitive than TST for diagnosis of active TB disease. Studies evaluating the incremental value of IGRAs to conventional microbiological tests show no meaningful contribution of IGRAs to the diagnosis of active TB. IGRAs are considered inadequate as rule-out or rule-in tests for active TB, especially in the context of HIV infection. IGRAs should not be used for the diagnosis of active TB disease" (WHO, 2015b).

The following recommendations involve LTBI (WHO, 2018).

- "Either a tuberculin skin test (TST) or interferon-gamma release assay (IGRA) can be used to test for LTBI."
- "LTBI testing by TST or IGRA is not a requirement for initiating preventive treatment in people living with HIV or child household contacts aged < 5 years. (Strong recommendation, moderate-quality evidence. Updated recommendation)"
- "Adults and adolescents living with HIV should be screened for TB according to a clinical algorithm. Those who do not report any of the symptoms of current cough, fever, weight loss or night sweats are unlikely to have active TB and should be offered preventive treatment, regardless of their ART status."
- "People living with HIV who have a positive test for LTBI benefit more from preventive treatment than those who have a negative LTBI test; LTBI testing can be used, where feasible, to identify such individuals."
- "Patients initiating anti-TNF treatment, patients receiving dialysis, patients preparing for an organ or haematological transplant and patients with silicosis should be systematically tested and treated for LTBI. (Strong recommendation, low–very low-quality evidence. Updated recommendation)"
- "In countries with a low TB incidence, systematic testing for and treatment of LTBI may be considered for prisoners, health workers, immigrants from countries with a high TB burden, homeless people and people who use illicit drugs. (Conditional recommendation, low–very low-quality evidence. Existing recommendation)"
- "Systematic testing for LTBI is not recommended for people with diabetes, people with harmful alcohol use, tobacco smokers and underweight people unless they are already included in the above recommendations. (Conditional recommendation, very low-quality evidence. Existing recommendation)"
- "There is no gold standard method for diagnosing LTBI. TST and IGRA require a competent immune response in order to identify people infected with TB and are imperfect tests for measuring progression to active disease" (WHO, 2018).

The WHO also published an additional guideline in 2020, which discusses preventive treatment. Some relevant recommendations and comments are listed below:

- "Either a tuberculin skin test (TST) or interferon-gamma release assay (IGRA) can be used to test for LTBI."
- "There is no strong evidence that one test should be preferred over the other in terms of predicting progression from TB infection to TB disease. Neither TSTs nor IGRAs should be used in persons having a low risk of TB infection and disease."
- A testing algorithm was also published in the guideline, which discusses latent TB testing and subsequent treatment in individuals at risk. The guideline writes that both asymptomatic household contacts (of patients with TB), as well as members of non-HIV risk groups (such as patients with "silicosis, dialysis, anti-TNF agent

treatment, preparation for transplantation or other risks in national guidelines" should be tested with TST or IGRA.

- "There is no gold standard method for diagnosing LTBI. TST and IGRA require a competent immune response in order to identify people infected with TB and are imperfect tests for measuring progression to active disease."

Finally, the WHO published an extensive guideline on the diagnosis of tuberculosis. Some relevant recommendations and comments are listed below:

- "Recommendations on Xpert MTB/RIF [Mycobacterium tuberculosis/rifampicin] and Xpert Ultra as initial tests in adults and children with signs and symptoms of pulmonary TB:

    1. In adults with signs and symptoms of pulmonary TB, Xpert MTB/RIF should be used as an initial diagnostic test for TB and rifampicin-resistance detection in sputum rather than smear microscopy/culture and phenotypic DST [drug susceptibility testing].
    (Strong recommendation, high certainty of evidence for test accuracy; moderate certainty of evidence for patient-important outcomes)
    2. In children with signs and symptoms of pulmonary TB, Xpert MTB/RIF should be used as an initial diagnostic test for TB and rifampicin-resistance detection in sputum, gastric aspirate, nasopharyngeal aspirate and stool rather than smear microscopy/culture and phenotypic DST.
    (Strong recommendation, moderate certainty for accuracy in sputum; low certainty of evidence for test accuracy in gastric aspirate, nasopharyngeal aspirate and stool)
    3. In adults with signs and symptoms of pulmonary TB and without a prior history of TB (≤5 years) or with a remote history of TB treatment (>5 years since end of treatment), Xpert Ultra should be used as an initial diagnostic test for TB and for rifampicin-resistance detection in sputum, rather than smear microscopy/culture and phenotypic DST.
    (Strong recommendation, high certainty of evidence for test accuracy)
    4. In adults with signs and symptoms of pulmonary TB and with a prior history of TB and an end of treatment within the last 5 years, Xpert Ultra may be used as an initial diagnostic test for TB and for rifampicin-resistance detection in sputum, rather than smear microscopy/culture and phenotypic DST.
    (Conditional recommendation, low certainty of evidence for test accuracy)
    5. In children with signs and symptoms of pulmonary TB, Xpert Ultra should be used as the initial diagnostic test for TB and detection of rifampicin resistance

in sputum or nasopharyngeal aspirate, rather than smear microscopy/culture and phenotypic DST.
(Strong recommendation, low certainty of evidence for test accuracy in sputum; very low certainty of evidence for test accuracy in nasopharyngeal aspirate)"

- "Recommendations on Xpert MTB/RIF and Xpert Ultra as initial tests in adults and children with signs and symptoms of extrapulmonary TB:

    6. In adults and children with signs and symptoms of TB meningitis, Xpert MTB/RIF or Xpert Ultra should be used in cerebrospinal fluid (CSF) as an initial diagnostic test for TB meningitis rather than smear microscopy/culture.
    (Strong recommendation, moderate certainty of evidence for test accuracy for Xpert MTB/RIF; low certainty of evidence for test accuracy for Xpert Ultra)
    7. In adults and children with signs and symptoms of extrapulmonary TB, Xpert MTB/RIF may be used in lymph node aspirate, lymph node biopsy, pleural fluid, peritoneal fluid, pericardial fluid, synovial fluid or urine specimens as the initial diagnostic test rather than smear microscopy/culture.
    (Conditional recommendation, moderate certainty of evidence for test accuracy for pleural fluid; low certainty for lymph node aspirate, peritoneal fluid, synovial fluid, urine; very low certainty for pericardial fluid, lymph nodes biopsy)
    8. In adults and children with signs and symptoms of extrapulmonary TB, Xpert Ultra may be used in lymph node aspirate and lymph node biopsy as the initial diagnostic test rather than smear microscopy/culture.
    (Conditional recommendation, low certainty of evidence)
    9. In adults and children with signs and symptoms of extrapulmonary TB, Xpert MTB/RIF or Xpert Ultra should be used for rifampicin-resistance detection rather than culture and phenotypic DST.
    (Strong recommendation, high certainty of evidence for test accuracy for Xpert MTB/RIF; low certainty of evidence for Xpert Ultra)
    10. In HIV-positive adults and children with signs and symptoms of disseminated TB, Xpert MTB/RIF may be used in blood, as an initial diagnostic test for disseminated TB.
    (Conditional recommendation, very low certainty of evidence for test accuracy)"

It should be noted that recommendation 10 (above) "applies only to a particular population (HIV-positive adults with signs and symptoms of disseminated TB). The GDG did not feel comfortable extrapolating this recommendation to other patient populations."

- "Recommendations on Xpert MTB/RIF and Xpert Ultra repeated testing in adults and children with signs and symptoms of pulmonary TB:

    11. In adults with signs and symptoms of pulmonary TB who have an Xpert Ultra trace positive result on the initial test, repeated testing with Xpert Ultra may not be used. (Conditional recommendation, very low certainty of evidence for test accuracy)
    12. In children with signs and symptoms of pulmonary TB in settings with pretest probability below 5% and an Xpert MTB/RIF negative result on the initial test, repeated testing with Xpert MTB/RIF in sputum, gastric fluid, nasopharyngeal aspirate or stool specimens may not be used.
    (Conditional recommendation, low certainty of evidence for test accuracy for sputum and very low for other specimen types)
    13. In children with signs and symptoms of pulmonary TB in settings with pretest probability 5% or more and an Xpert MTB/RIF negative result on the initial test, repeated testing with Xpert MTB/RIF (for total of two tests) in sputum, gastric fluid, nasopharyngeal aspirate and stool specimens may be used.
    (Conditional recommendation, low certainty of evidence for test accuracy for sputum and very low for other specimen types)
    14. In children with signs and symptoms of pulmonary TB in settings with pretest probability below 5% and an Xpert Ultra negative result on the initial test, repeated testing with Xpert Ultra in sputum or nasopharyngeal aspirate specimens may not be used.
    (Conditional recommendation, very low certainty of evidence for test accuracy)
    15. In children with signs and symptoms of pulmonary TB in settings with pretest probability 5% or more and an Xpert Ultra negative result on the first initial test, repeated one Xpert Ultra test (for a total of two tests) in sputum and nasopharyngeal aspirate specimens may be used.
    (Conditional recommendation, very low certainty of evidence for test accuracy)"

- "Recommendations on Xpert MTB/RIF and Xpert Ultra as initial tests for pulmonary TB in adults in the general population either with signs and symptoms of TB or chest radiograph with lung abnormalities or both:

    16. In adults in the general population who had either signs or symptoms of TB or chest radiograph with lung abnormalities or both, the Xpert MTB/RIF or Xpert Ultra may replace culture as the initial test for pulmonary TB.

(Conditional recommendation, low certainty of the evidence in test accuracy for Xpert)

17. In adults in the general population who had either a positive TB symptom screen or chest radiograph with lung abnormalities or both, one Xpert Ultra test may be used rather than two Xpert Ultra tests as the initial test for pulmonary TB.
    (Conditional recommendation, very low certainty of evidence for test accuracy)"

It should be noted that recommendation 16 (above) "applies only to the use of Xpert MTB/RIF or Xpert Ultra for clinical case management in situations where an immediate decision on patient treatment needs to be made and recourse to supplementary tests is not available or would incur delays." Moreover, recommendation 17 (above) "applies only to the use of Xpert Ultra for clinical case management."

- "Recommendations on Truenat MTB, MTB Plus, and Truenat MTB-RIF Dx in adults and children with signs and symptoms of pulmonary TB:
  1. In adults and children with signs and symptoms of pulmonary TB, the Truenat MTB or MTB Plus may be used as an initial diagnostic test for TB rather than smear microscopy/culture.
     (Conditional recommendation, moderate certainty of evidence for test accuracy)
  2. In adults and children with signs and symptoms of pulmonary TB and a Truenat MTB or MTB Plus positive result, Truenat MTB-RIF Dx may be used as an initial test for rifampicin resistance rather than culture and phenotypic DST.
     (Conditional recommendation, very low certainty of evidence for test accuracy)"

Recommendation 1 is "is extrapolated to children for sputum, although the tests are expected to be less sensitive in children."

- Regarding first-line LPAs [line probe assays]:
  - "For persons with a sputum smear-positive specimen or a cultured isolate of MTBC, commercial molecular LPAs may be used as the initial test instead of phenotypic culture-based DST to detect resistance to rifampicin and isoniazid."
    (Conditional recommendation, moderate certainty in the evidence for the test's accuracy)

The WHO clarifies the above recommendation with the following remarks:

1. These recommendations apply to the use of LPAs for testing sputum smear-positive specimens (direct testing) and cultured isolates of MTBC (indirect testing) from both pulmonary and extrapulmonary sites.
2. LPAs are not recommended for the direct testing of sputum smear-negative specimens.
3. These recommendations apply to the detection of MTBC and the diagnosis of MDR-TB, but acknowledge that the accuracy of detecting resistance to rifampicin and isoniazid differs and, hence, that the accuracy of a diagnosis of MDR-TB is reduced overall.
4. These recommendations do not eliminate the need for conventional culture-based DST, which will be necessary to determine resistance to other anti-TB agents and to monitor the emergence of additional drug resistance.
5. Conventional culture-based DST for isoniazid may still be used to evaluate patients when the LPA result does not detect isoniazid resistance. This is particularly important for populations with a high pretest probability of resistance to isoniazid.
6. These recommendations apply to the use of LPA in children based on the generalization of data from adults. (WHO, 2021)

- Regarding second-line LPAs (SL-LPA):

1. "For patients with confirmed MDR/RR-TB [multi-drug resistant/rifampicin-resistant tuberculosis], SL-LPA may be used as the initial test, instead of phenotypic culture-based DST, to detect resistance to fluoroquinolones.
2. For patients with confirmed MDR/RR-TB, SL-LPA may be used as the initial test, instead of phenotypic culture-based DST, to detect resistance to the SLIDs [second-line injectable drug]."

- Regarding Lateral flow urine lipoarabinomannan assay [LF-LAM]:

"**In inpatient settings**

1. WHO strongly recommends LF-LAM to assist in the diagnosis of active TB in HIV-positive adults, adolescents and children:

1.1 with signs and symptoms of TB (pulmonary and/or extrapulmonary) or seriously ill
(**strong** recommendation, moderate certainty in the evidence about the intervention effects); or

1.2 with advanced HIV disease or who are seriously ill
(**strong** recommendation, moderate certainty in the evidence about the intervention effects); or

1.3 irrespective of signs and symptoms of TB and with a CD4 cell count of less than 200 cells/mm^3
(**strong** recommendation, moderate certainty in the evidence about intervention effects)

In outpatient settings

2. WHO suggests using LF-LAM to assist in the diagnosis of active TB in HIV-positive adults, adolescents and children:

2.1 with signs and symptoms of TB (pulmonary and/or extrapulmonary) or seriously ill
(**conditional** recommendation, low certainty in the evidence about test accuracy);

2.2 irrespective of signs and symptoms of TB and with a CD4 cell count of less than 100 cells/mm^3
(**conditional** recommendation, very low certainty in the evidence about test accuracy)

In outpatient settings

3. WHO recommends **against** using LF-LAM to assist in the diagnosis of active TB in HIV-positive adults, adolescents, and children:

3.1 without assessing TB symptoms (**strong** recommendation, very low certainty in the evidence about test accuracy);

3.2 without TB symptoms and unknown CD4 cell count or without TB symptoms and CD4 cell count greater than or equal to 200 cells/mm^3
(**strong** recommendation, very low certainty in the evidence about test accuracy); and

3.3 without TB symptoms and with a CD4 cell count of 100-200 cells/mm^3
(**conditional** recommendation, very low certainty in the evidence about test accuracy)."

Finally, WHO did not discuss whole genome sequencing of clinical isolates in the context of assessing drug resistance susceptibility for TB (WHO, 2021).

American Thoracic Society/Infectious Diseases Society of America/Centers for Disease Control and Prevention

The ATS/IDSA/CDC published clinical practice guidelines for diagnosis of TB in 2017 that stated the following:

LTBI:

- "We recommend performing an interferon-γ release assay (IGRA) rather than a tuberculin skin test (TST) in individuals 5 years or older who meet the following criteria: (1) are likely to be infected with *Mtb*, (2) have a low or intermediate risk of disease progression, (3) it has been decided that testing for LTBI is warranted, and (4) either have a history of BCG vaccination or are unlikely to return to have their TST read (*strong recommendation, moderate-quality evidence*)."
- "We suggest performing an IGRA rather than a TST in all other individuals 5 years or older who are likely to be infected with *Mtb*, who have a low or intermediate risk of disease progression, and in whom it has been decided that testing for LTBI is warranted (*conditional recommendation, moderate-quality evidence*)."
- "There are insufficient data to recommend a preference for either a TST or an IGRA as the first-line diagnostic test in individuals 5 years or older who are likely to be infected with *Mtb*, who have a high risk of progression to disease, and in whom it has been determined that diagnostic testing for LTBI is warranted."
- "Guidelines recommend that persons at low risk for *Mtb* infection and disease progression NOT be tested for *Mtb* infection. We concur with this recommendation. However, we also recognize that such testing may be obliged by law or credentialing bodies. If diagnostic testing for LTBI is performed in individuals who are unlikely to be infected with *Mtb* despite guidelines to the contrary:"
    - "We suggest performing an IGRA instead of a TST in individuals 5 years or older (*conditional recommendation, low-quality evidence*). Remarks: A TST is an acceptable alternative in settings where an IGRA is unavailable, too costly, or too burdensome."
    - "We suggest a second diagnostic test if the initial test is positive in individuals 5 years or older (*conditional recommendation, very low-quality evidence*). Remarks: The confirmatory test may be either an IGRA or a TST. When such testing is performed, the person is considered infected only if both tests are positive."

- "We suggest performing a TST rather than an IGRA in healthy children <5 years of age for whom it has been decided that diagnostic testing for LTBI is warranted (*conditional recommendation, very low-quality evidence*)."
- "While both IGRA and TST testing provide evidence for infection with Mtb, they cannot distinguish active from latent TB. Therefore, the diagnosis of active TB must be excluded prior to embarking on treatment for LTBI. This is typically done by determining whether or not symptoms suggestive of TB disease are present, performing a chest radiograph and, if radiographic signs of active TB (eg, airspace opacities, pleural effusions, cavities, or changes on serial radiographs) are seen, then sampling is performed, and the patient managed accordingly."

TB Disease:

- "We recommend that acid-fast bacilli (AFB) smear microscopy be performed, rather than no AFB smear microscopy, in all patients suspected of having pulmonary TB."
- "We suggest that both liquid and solid mycobacterial cultures be performed, rather than either culture method alone, for every specimen obtained from an individual with suspected TB disease."
- "We suggest performing a diagnostic nucleic acid amplification test (NAAT), rather than not performing a NAAT, on the initial respiratory specimen from patients suspected of having pulmonary TB."
- "We recommend performing rapid molecular drug susceptibility testing for rifampin with or without isoniazid using the respiratory specimens of persons who are either AFB smear positive or Hologic Amplified MTD positive and who meet one of the following criteria: (1) have been treated for tuberculosis in the past, (2) were born in or have lived for at least 1 year in a foreign country with at least a moderate tuberculosis incidence (≥20 per 100000) or a high primary multidrug-resistant tuberculosis prevalence (≥2%), (3) are contacts of patients with multidrug-resistant tuberculosis, or (4) are HIV infected."
- "We suggest mycobacterial culture of respiratory specimens for all children suspected of having pulmonary TB."
- "We suggest that cell counts, and chemistries be performed on amenable fluid specimens collected from sites of suspected extrapulmonary TB.
- "We suggest that adenosine deaminase levels be measured, rather than not measured, on fluid collected from patients with suspected pleural TB, TB meningitis, peritoneal TB, or pericardial TB."
- "We suggest that free IFN-γ levels be measured, rather than not measured, on fluid collected from patients with suspected pleural TB or peritoneal TB."

- "We suggest that AFB smear microscopy be performed, rather than not performed, on specimens collected from sites of suspected extrapulmonary TB.
- "We recommend that mycobacterial cultures be performed, rather than not performed, on specimens collected from sites of suspected extrapulmonary TB."
- "We suggest that NAAT be performed, rather than not performed, on specimens collected from sites of suspected extrapulmonary TB."
- "We suggest that histological examination be performed, rather than not performed, on specimens collected from sites of suspected extrapulmonary TB."
- "Recently, whole-genome sequencing (WGS) has been applied to investigation of tuberculosis outbreaks. This technique may add discriminatory power to strain identification, but the role of WGS in outbreak investigation is still being determined."
- "We recommend one culture isolate from each mycobacterial culture-positive patient be submitted to a regional genotyping laboratory for genotyping" (Lewinsohn et al., 2017).

National Institute of Health (NIH)

The NIH published a set of guidelines regarding opportunistic infections in HIV-positive patients. The NIH writes that "All persons with HIV should be tested for LTBI [latent TB infection] at the time of HIV diagnosis, regardless of their epidemiological risk of TB exposure."

The NIH also comments on diagnostic testing, stating that "sputum acid-fast bacilli (AFB) smear, nucleic acid amplification (NAA) testing, and AFB culture should be performed in people with HIV with symptoms of TB disease who have a normal chest radiograph, as well as in those with no pulmonary symptoms but evidence of TB disease elsewhere in the body." The NIH remarks that "pleural fluid, pericardial fluid, ascites, and cerebrospinal fluid should be sampled if clinical evidence of involvement exists."

The NIH also discusses drug resistance testing, recommending that "Drug resistance should be considered in all people with HIV, especially those who meet any of the following criteria:

- Known exposure to a person with drug-resistant TB,
- Residence in a setting with high rates of primary drug-resistant TB,
- Persistently positive smear or culture results at or after four months of treatment, or

- Previous TB treatment, particularly if it was not directly observed or was interrupted for any reason."

The NIH recommends "Rapid molecular DST for rifampin (and isoniazid, if available) should be performed on the initial isolates from all patients suspected of having TB, because resistance to rifampin is associated with an increased risk of treatment failure, recurrent TB, and amplification of resistance to additional TB medications."

Overall, the NIH recommends that "For all patients with TB disease, phenotypic DST to first-line TB drugs (isoniazid, rifampin, ethambutol, and pyrazinamide) should be performed, regardless of the source of the specimen. Given the alternative of a shorter drug-susceptible TB regimen containing moxifloxacin, public health laboratories in the U.S. may add routine moxifloxacin susceptibility testing as well. Molecular resistance testing should be performed, and resistance testing should be repeated if sputum cultures remain positive for M. tuberculosis at or after 4 months of treatment or become positive again 1 month or longer after culture conversion to negative. Resistance testing for second-line TB medications (including bedaquiline, linezolid, clofazimine, pretomanid, cycloserine, ethionamide, and others) should be limited to specimens with resistance to first-line TB medications and should be performed in reference laboratories with substantial experience in these techniques." The NIH makes a further stipulation that "isolates with an initial reading of rifampin by commercial NAA test should undergo confirmatory testing (rpoB gene sequencing and phenotypic DST). Clinicians who suspect drug-resistant TB in a patient with HIV should make every effort to expedite a diagnosis and consult with their state TB program and then the CDC as needed" (NIH, 2024).

American Thoracic Society, U.S. Centers for Disease Control and Prevention, European Respiratory Society, and Infectious Diseases Society of America (ATS/CDC/ERS/IDSA)

This joint guideline was published to discuss the treatment of drug-resistant tuberculosis. The guideline notes that "molecular DSTs [drug susceptibility tests] should be obtained for rapid detection of mutations associated with resistance. When rifampin resistance is detected, additional DST should be performed immediately for first-line drugs, fluoroquinolones, and aminoglycosides." The guideline further stated that "A rapid test for a [sic] least rifampin resistance should ideally be done for every patient, but especially for those at risk of drug resistance." Individuals who "have or recently had close contact with a patient with infectious DR-TB [drug resistant tuberculosis] especially when the contact is a young child or has HIV infection, are at risk of developing DR-TB."

The guideline also remarks that if "sputum cultures remain positive after 3 months of treatment, or if there is bacteriological reversion from negative to positive at any time, DST [drug susceptibility testing] should be repeated" and that monthly cultures help to "identify early evidence of failure." Finally, this guideline refers to the above 2017 Lewinsohn guideline as providing "additional details on the optimal use of diagnostic tools and algorithms" (Nahid et al., 2019).

United State Preventative Service Task Force (USPSTF)

A 2023 recommendation from USPSTF found adequate evidence that accurate screening tests for LTBI are available, that the treatment of LTBI provides a moderate health benefit in preventing progression to active disease, and that the harms of screening and treatment are small. The USPSTF has moderate certainty that screening for LTBI in persons at increased risk for infection provides a moderate net benefit. "This recommendation applies to asymptomatic adults 18 years or older at increased risk for tuberculosis (TB). It does not apply to adults with symptoms of TB or to children and adolescents" (USPSTF et al., 2023).

The USPSTF also notes that to achieve the benefit of this screening, it is important that persons who screen positive for LTBI receive follow-up and treatment. While the USPSTF found no evidence on the optimal frequency of screening for LTBI, in the absence of evidence, they recommend that "a reasonable approach is to repeat screening based on specific risk factors; screening frequency could range from 1-time-only screening among persons at low risk for future TB exposure to annual screening among those who are at continued risk of exposure (USPSTF et al., 2023).

The USPSTF provides additional information on how to implement their recommendation:

- "Populations at increased risk for LTBI, based on increased prevalence of active disease and increased risk of exposure, include persons who were born in, or are former residents of, countries with high TB prevalence and persons who live in, or have lived in, high-risk congregate settings (e.g., homeless shelters or correctional facilities).
- Clinicians can consult their local or state health departments for more information about populations at increased risk in their community, since local demographic patterns may vary across the US.
- Two types of screening tests for LTBI are currently available in the US: the tuberculin skin test (TST) and the interferon-gamma release assay (IGRA).
    - The TST requires trained personnel to administer intradermal purified protein derivative and interpret the response 48 to 72 hours later.

- The IGRA requires a single venous blood sample that measures the CD4 T-cell response to specific Mycobacterium tuberculosis antigens and laboratory processing within 8 to 30 hours after collection.
- Testing with IGRA may have advantages over TST for persons who have received a BCG vaccination, as IGRA does not cross-react with the vaccine, and for persons who may be unlikely to return for TST interpretation" (USPSTF et al., 2023).

The USPSTF provides the following additional information for clinicians to know pertaining to their recommendation:

- "TB disproportionately affects Asian, Black, Hispanic/Latino, Native American/Alaska Native, and Native Hawaiian/Pacific Islander persons. Incidence of TB varies by geography and living accommodations, suggesting an association with social determinants of health.
- LTBI is an infection with M tuberculosis in which the bacteria are alive but contained by the immune system. Persons with LTBI have no apparent symptoms, do not feel sick, cannot spread TB to others, and usually have a positive TB skin test or positive TB blood test reaction.
- Active TB or TB disease is an illness in which TB bacteria are multiplying and attacking a part of the body, usually the lungs. TB disease may be symptomatic (including weakness, weight loss, fever, no appetite, chills, sweating at night, bad cough, pain in the chest, or coughing up blood). A person with TB disease may be infectious and spread TB bacteria to others" (USPSTF et al., 2023).

Infectious Diseases Society of America (IDSA)/American Society of Microbiology (ASM)

In the 2024 update to the IDSA/ASM joint guideline, A Guide to Utilization of the Microbiology Laboratory for Diagnosis of Infectious Diseases, concerning Mycobacterium tuberculosis, they recommend AFB smear or AFB culture when performing laboratory diagnosis. They do allow for the use of NAAT for diagnosing M. tuberculosis; however, they state, "A negative result does not rule out Mycobacterium tuberculosis." They also state "although most molecular tests have excellent sensitivity, a *Mycobacterium tuberculosis NAAT* test should be an adjunct to a culture and never ordered alone. No current commercial methods are FDA-cleared for these specimens, so laboratories must have validated the test they use."

In cases of laboratory diagnosis of pulmonary infections in cystic fibrosis due to suspected *Mycobacterium* spp, they recommend performing a mycobacterial culture from the expectorated sputum, bronchoscopically obtained cultures, or other respiratory cultures (Miller et al., 2024).

Committee on Infectious Diseases, American Academy of Pediatrics, 32nd Edition (2021-2024, Red Book)

Highlights from the updated Red Book include the following:

- The AAP notes that there are NAATs cleared by the FDA for detection of *M. tuberculosis* from smear-positive and smear-negative sputum specimens.
- For children younger than two years, the TST [tuberculin skin test] is the preferred method for detection of infection.
- "For children 2 years and older, either TST or IGRA [interferon gamma release assay] can be used, but in people previously vaccinated with BCG, IGRA is preferred to avoid a false-positive TST result caused by a previous vaccination with BCG."
- Universal testing with either TST or IGRA is discouraged "because it results in either a low yield of positive results or a large proportion of false-positive results, leading to an inefficient use of health care resources."
- All organ transplant candidates should be given a TST or IGRA before starting immunosuppressive therapy.
- The AAP recommends the following for an "immediate" TST or IGRA:
  - children who are contacts of people with confirmed or suspected contagious tuberculosis (contact investigation)
  - children with clinical or radiographic findings suggesting TB
  - children immigrating from countries with endemic infection (e.g., Asia, Middle East, Africa, Latin America, countries of the former Soviet Union), including international adoptees
  - children with history of significant travel to countries with endemic infection who have substantial contact with the resident population
- The AAP also recommends an annual TST/IGRA for children with HIV (AAP, 2021).

Tuberculosis Network European Trials Group (TBNET)/RESIST-TB

This consensus statement encompasses molecular drug resistance testing for *Mycobacterium tuberculosis*.

- "Although they do not cover all mutations involved in RMP resistance, molecular methods for RMP could be considered a standard for the diagnostic evaluation of patients with presumptive MDR-TB. In low MDR-TB prevalence countries, physicians should be aware of possible false-positive resistance results of molecular tests, and RMP resistance should be confirmed by a second molecular test on a different sample or by phenotypic tests."
- "Although >90% of RMP-resistant strains are also resistant to INH, molecular testing for INH drug resistance is important."

- "In all patients with evidence of M. tuberculosis with an rpoB mutation in a direct specimen or when DST indicates MDR-TB, molecular testing for second-line resistance should be undertaken to guide treatment and to reduce the time to diagnose XDR-TB."
- "WGS [whole genome sequencing] provides the complete sequence information of the bacterial genome. However, due to the lack of correlation with in vitro (phenotypic DST) and in vivo (treatment outcome) data at present, it is not possible to interpret the clinical value of the vast majority of mutations or polymorphisms detected."
- "The level of discordance between molecular and culture-based DST depends on the drug and the genomic region evaluated. Despite the fact that results of phenotypic methods do not always correspond to response to clinical treatment, culture-based methods are still regarded by most experts involved in this document as the gold standard for DST" (Domínguez et al., 2016).

National Institute for Health and Care Excellence (NICE)

NICE has published guidelines for assessment of TB, which include the following recommendations:

- "If the Mantoux test is positive but a diagnosis of active TB is excluded, consider an interferon gamma release assay if more evidence of infection is needed to decide on treatment."
- "For adults who are severely immunocompromised, such as those with HIV and CD4 counts of fewer than 200 cells/mm^3, or after solid organ or allogeneic stem cell transplant, offer an interferon-gamma release assay and a concurrent Mantoux test."
- "For other adults who are immunocompromised, consider an interferon-gamma release assay alone or an interferon-gamma release assay with a concurrent Mantoux test. If either test is positive (for Mantoux, this is an induration of 5 mm or larger, regardless of BCG history), assess for active TB."
- "Only consider using interferon-gamma release assays alone in children and young people if Mantoux testing is not available or is impractical."
- "If TB is a possibility, microbiology staff should consider carrying out TB culture on samples, even if it is not requested."
- "Request rapid diagnostic nucleic acid amplification tests for the *M. tuberculosis* complex (*M. tuberculosis, M. bovis, M. africanum*) on primary specimens if there is clinical suspicion of TB disease, and:
    - the person has HIV or
    - rapid information about mycobacterial species would alter the person's care or

    - the need for a large contact-tracing initiative is being explored."
- "For people with clinically suspected TB, a TB specialist should request rapid diagnostic nucleic acid amplification tests for rifampicin resistance on primary specimens if a risk assessment for multidrug resistance identifies any of the following risk factors:
    - "history of previous TB drug treatment, particularly if there was known to be poor adherence to that treatment"
    - "contact with a known case of multidrug-resistant TB"
    - "birth or residence in a country in which the World Health Organization reports that a high proportion (5% or more) of new TB cases are multidrug-resistant."
- If the rapid diagnostic nucleic acid amplification test for the *M. tuberculosis* complex is negative in a person at high risk of multidrug-resistant TB:
    - "obtain further specimens for nucleic acid amplification testing and culture, if possible"
    - "use rapid rifampicin resistance detection on cultures that become positive for the *M. tuberculosis* complex"
- "If the rapid diagnostic nucleic acid amplification test for rifampicin resistance is positive:
    - "test for resistance to second-line drugs" (NICE, 2024).

European Respiratory Society (ERS) and the European Centre for Disease Prevention and Control (ECDC) Statement: European Union Standards for Tuberculosis Care

This joint guideline was intended to "define the essential level of care for managing patients who have or are presumed to have TB, or are at increased risk of developing the disease."

- "All patients (adults, adolescents and children who are capable of producing sputum) thought to have pulmonary tuberculosis should have at least two sputum specimens submitted for microscopic examination and one for rapid testing for the identification of tuberculosis and drug resistance using an internationally recommended (rapid) molecular test. The sample should be sent for liquid culture and, if positive, for culture-based drug susceptibility testing (DST) in a quality-assured laboratory."
- "For all patients (adults, adolescents and children) presumed to have extrapulmonary tuberculosis, appropriate specimens from the suspected sites of involvement should be obtained for microbiological testing (microscopy, rapid molecular tests, culture, species identification, DST with rapid molecular tests and

culture-based techniques) and histopathological examination in quality-assured laboratories."

- "All persons with chest radiographic findings suggestive of pulmonary tuberculosis should have sputum specimens submitted for microscopic examination, rapid molecular tests, culture, species identification and DST with rapid molecular tests and culture-based techniques in a quality-assured laboratory" (ERS/ECDC, 2017).

National Society of Tuberculosis Clinicians (NSTC) of the National Tuberculosis Controllers Association (NTCA)

In 2023, the NSTC of the NTCA jointly released a set of clinical recommendations for "Testing and Treatment of Latent Tuberculosis Infection in the United States." In relation to testing, the NTSC/NTCA states that "IGRAs are generally preferred, but the TST is acceptable… In choosing which test to use, consider the patient's history of BCG, age, and ability to return for a second appointment. IGRAs offer greater specificity than a TST in persons who were BCG vaccinated or who have non-tuberculous mycobacterial infections. For this reason, IGRAs are preferred for most non-US-born patients who received, or may have received, BCG vaccination. For other persons, either a TST or IGRA can be used depending on test availability and cost."

When discussing immunocompromised patients, the organizations stated that "dual testing with TST and an IGRA simultaneously increases the overall specificity for infection." However, "Dual testing should not be routine, but it may be considered for patients when there is concern about their ability to mount a strong immune response to a test, for persons who are at risk of severe forms of TB disease, or for persons in whom TB infection is strongly suspected because of exposure risks or symptomatology. Children aged <2 years old can be included in a dual testing strategy if one of the above circumstances is present."

In regard to serial testing, "When serial or periodic testing is required, as with some health care personnel at ongoing risk for TB exposure, either an IGRA or the TST may be used. For TST testing, the initial test should be a two-step TST. Because IGRAs do not cause boosting, serial testing with IGRAs does not require two-step testing to establish a baseline."

For persons who are "at low risk for TB infection or active TB disease are required to be tested by law for other reasons, use either an IGRA or TST. If the result is positive, perform a second test with the same or a different method to confirm the test result."

When an MMR vaccine and TB test are both indicated, the Advisory Committee on Immunization Practices recommends:

- "Administer the TST or IGRA simultaneously with the live vaccine (preferred scenario).
- If a TST or IGRA has already been administered, a live vaccine can be administered at any time >1 day after the administration of the TB test.
- If a live vaccine has already been administered, wait at least 28 days before administering a TST or IGRA.
- In two-step testing, wait at least 28 days after the live vaccine is administered before administering the first TST. Continue from there to complete the two-step testing. Wait to administer any additional doses of live vaccine until after the second TST is measured."

In terms of officially diagnosing latent tuberculosis infection, the NSTC states, "At the completion of pretreatment clinical evaluation, if a patient with a positive test result for TB infection does not have any symptoms of TB, and the CXRs and other diagnostic tests results are normal, then active TB disease is excluded and LTBI is diagnosed" (NSTC, 2023).

National Psoriasis Foundation (NPF)

In reviewing the literature surrounding immunosuppressive therapies and the risk of tuberculosis, the National Psoriasis Foundation found that "The biologic TNF–α inhibitors are very promising in the treatment of psoriasis. However, because TNF–α is also an important cytokine in preventing TB infection and in keeping latent TB infection from becoming active disease, the use of TNF–α inhibitors has been associated with an increased risk of developing active TB. A higher incidence of TB has also been reported with other immunosuppressive/immunomodulatory treatments for psoriasis. It is, therefore, of utmost importance to appropriately screen all patients for latent TB infection prior to initiating any immunologic therapy. Delaying immunologic therapy until latent TB infection prophylaxis is completed is preferable. However, if the patient is adhering to his prophylactic regimen and is appropriately tolerating the regimen, therapy may be started after one to two months if the clinical condition requires" (Doherty et al., 2008). This screening "for latent TB infection before commencement of treatment is of utmost importance when beginning treatment with the tumor necrosis factor–α inhibitors, T-cell blockers, cyclosporine, or methotrexate" and the "currently recommended method for screening is the tuberculin skin test." However, the authors also acknowledge that "There are few evidence-based studies on screening for latent TB infection in psoriasis patients treated with systemic and biologic agents," and so the power of the results may be limited (Doherty et al., 2008).

Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
81099	Unlisted urinalysis procedure
81425	Genome (eg, unexplained constitutional or heritable disorder or syndrome); sequence analysis
81426	Genome (eg, unexplained constitutional or heritable disorder or syndrome); sequence analysis, each comparator genome (eg, parents, siblings) (list separately in addition to code for primary procedure)
81479	Unlisted molecular pathology procedure
82945	Glucose, body fluid, other than blood
83520	Immunoassay for analyte other than infectious agent antibody or infectious agent antigen; quantitative, not otherwise specified
83615	Lactate dehydrogenase (LD), (LDH)
84157	Protein, total, except by refractometry; other source (eg, synovial fluid, cerebrospinal fluid)
84311	Spectrophotometry, analyte not elsewhere specified
86480	Tuberculosis test, cell mediated immunity antigen response measurement; gamma interferon
86481	Tuberculosis test, cell mediated immunity antigen response measurement; enumeration of gamma interferon-producing T-cells in cell suspension

87070	Culture, bacterial; any other source except urine, blood or stool, aerobic, with isolation and presumptive identification of isolates
87077	Culture, bacterial; aerobic isolate, additional methods required for definitive identification, each isolate
87116	Culture, tubercle or other acid-fast bacilli (eg, TB, AFB, mycobacteria) any source, with isolation and presumptive identification of isolates
87149	Culture, typing; identification by nucleic acid (DNA or RNA) probe, direct probe technique, per culture or isolate, each organism probed
87150	Culture, typing; identification by nucleic acid (DNA or RNA) probe, amplified probe technique, per culture or isolate, each organism probed
87153	Culture, typing; identification by nucleic acid sequencing method, each isolate (eg, sequencing of the 16S rRNA gene)
87181	Susceptibility studies, antimicrobial agent; agar dilution method, per agent (eg, antibiotic gradient strip)
87184	Susceptibility studies, antimicrobial agent; disk method, per plate (12 or fewer agents)
87185	Susceptibility studies, antimicrobial agent; enzyme detection (eg, beta lactamase), per enzyme
87186	Susceptibility studies, antimicrobial agent; microdilution or agar dilution (minimum inhibitory concentration [MIC] or breakpoint), each multi-antimicrobial, per plate
87187	Susceptibility studies, antimicrobial agent; microdilution or agar dilution, minimum lethal concentration (MLC), each plate (list separately in addition to code for primary procedure)
87188	Susceptibility studies, antimicrobial agent; macrobroth dilution method, each agent
87190	Susceptibility studies, antimicrobial agent; mycobacteria, proportion method, each agent
87206	Smear, primary source with interpretation; fluorescent and/or acid fast stain for bacteria, fungi, parasites, viruses or cell types
87550	Infectious agent detection by nucleic acid (DNA or RNA); Mycobacteria species, direct probe technique
87551	Infectious agent detection by nucleic acid (DNA or RNA); Mycobacteria species, amplified probe technique
87552	Infectious agent detection by nucleic acid (DNA or RNA); Mycobacteria species, quantification
87555	Infectious agent detection by nucleic acid (DNA or RNA); Mycobacteria tuberculosis, direct probe technique

87556	Infectious agent detection by nucleic acid (DNA or RNA); Mycobacteria tuberculosis, amplified probe technique
87557	Infectious agent detection by nucleic acid (DNA or RNA); mycobacteria tuberculosis, quantification
87560	Infectious agent detection by nucleic acid (DNA or RNA); mycobacteria avium-intracellulare, direct probe technique
87561	Infectious agent detection by nucleic acid (DNA or RNA); mycobacteria avium-intracellulare, amplified probe technique
87562	Infectious agent detection by nucleic acid (DNA or RNA); mycobacteria avium-intracellulare, quantification

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

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Revision History

Revision Date	Summary of Changes
09/04/2024	Reviewed and Updated: Updated the background, guidelines and recommendations, and evidence-based scientific references. Literature review did not necessitate any modifications to coverage criteria. The following edits were made for clarity and consistency: Verb tense in CC13 and CC14 fixed from “testing DO not meet” to “testing DOES not meet”.

Testing for Vector-Borne Infections

Policy Number: AHS – G2158 – Testing for Vector-borne Infections	Prior Policy Name and Number, as applicable: AHS – G2158 – Testing for Mosquito- or Tick-Related Infections
Initial Presentation Date: 09/25/2018 Effective Date: February 1, 2025	

Policy Description

Arthropod vectors, including mosquitoes, ticks, fleas, and mites, that feed on vertebrate hosts can spread bacteria, protozoa, and viruses during feeding to their susceptible host, resulting in a variety of infections and diseases. Arboviruses (arthropod-borne viruses) include Zika virus, West Nile virus (WNV), chikungunya virus, dengue virus (DENV), yellow fever virus (YFV), and Colorado tick fever virus (CTF) to name a few. Malaria and babesiosis are both conditions caused by arthropod-borne protozoan parasites, Plasmodium and Babesia, respectively. Conditions caused by arthropod-borne bacteria include rickettsial diseases, ehrlichiosis, anaplasmosis, and Lyme disease, as well as other Borrelia-associated disorders (Calisher, 1994; CDC, 2024s). Isolation, identification, and characterization of these various infections depend on the causative agent. Identification methods may include culture testing, microscopy, and staining techniques; moreover, molecular testing, such as nucleic acid amplification testing (NAAT), and serologic testing, including immunofluorescence antibody assays and enzyme-linked immunosorbent assays (ELISA), can be used for laboratory diagnosis (Miller et al., 2024).

For Lyme disease and testing for *Borrelia burgdorferi*, please see AHS-G2143 Lyme Disease Testing.

Related Policies

Policy Number	Policy Title
AHS-G2143	Lyme Disease Testing
AHS-M2097	Identification of Microorganisms Using Nucleic Acid Probes

Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) For individuals suspected of having babesiosis (see Note 1), the use of a Giemsa- or Wright-stain of a blood smear **or** nucleic acid amplification testing (NAAT) **MEETS COVERAGE CRITERIA**.

2) For individuals suspected of having babesiosis (see Note 1), the use of either an IgG or IgM indirect immunofluorescence antibody (IFA) assay for Babesia **DOES NOT MEET COVERAGE CRITERIA**.

3) For individuals suspected of having a relapsing fever caused by a *Borrelia* spp., the following testing **MEETS COVERAGE CRITERIA**:

   a) For individuals suspected of having hard tick relapsing fever (HTRF) (see Note 2): serologic assays to detect *Borrelia* antibodies or PCR testing to detect *Borrelia miyamotoi*.

   b) For individuals suspected of having louse-borne relapsing fever (LBRF) (see Note 3): peripheral blood smear microscopy or PCR testing to detect *Borrelia recurrentis*.

   c) For individuals suspected of having a soft tick relapsing fever (STRF)/tickborne relapsing fever (TBRF) (see Note 4): dark-field microscopy of a peripheral blood smear, microscopy of a Wright- or Giemsa-stained blood smear, PCR testing to detect *Borrelia* spp., or serologic assays to detect *Borrelia* antibodies.

4) For individuals suspected of having a relapsing fever caused by a *Borrelia* spp., culture testing for *Borrelia* **DOES NOT MEET COVERAGE CRITERIA**.

5) For individuals suspected of having chikungunya (see Note 5), the use of viral culture for diagnosis, NAAT for the presence of chikungunya in a serum sample, or IFA assay for IgM antibodies during both the acute and convalescent phases **MEETS COVERAGE CRITERIA**.

6) For individuals suspected of having Colorado tick fever (CTF) (see Note 6), the use of PCR testing or IFA for CTF-specific IgM antibodies **MEETS COVERAGE CRITERIA**.

7) For the detection of dengue virus (DENV), the use of NAAT, IgM antibody capture ELISA (MAC-ELISA), **or** NS1 ELISA, as well as a confirmatory plaque reduction neutralization test for DENV, **MEETS COVERAGE CRITERIA** in the following individuals:

   a) For individuals suspected of having a DENV infection (see Note 7).

   b) For individuals who are symptomatic for Zika virus infection (see Note 8).

8) For individuals suspected of having DENV (see Note 7), the use of IgG ELISA **or** hemagglutination testing **DOES NOT MEET COVERAGE CRITERIA**.

9) For individuals suspected of having ehrlichiosis and/or anaplasmosis (see Note 8), the use of NAAT of whole blood, IFA assay for IgG antibodies, or microscopy for morulae detection **MEETS COVERAGE CRITERIA**.

10) For individuals suspected of having ehrlichiosis and/or anaplasmosis (see Note 8), the use of an IFA assay for IgM antibodies **or** standard blood culture **DOES NOT MEET COVERAGE CRITERIA**.

11) For individuals suspected of having malaria (see Note 10), the use of a rapid immunochromatographic diagnostic test **or** smear microscopy to diagnose malaria, determine the species of *Plasmodium*, identify the parasitic life-cycle stage, and/or quantify the parasitemia (can be repeated up to three times within three days if initial microscopy is negative in suspected cases of malaria) **MEETS COVERAGE CRITERIA**.

12) To confirm the species of *Plasmodium* in an individual diagnosed with malaria, PCR testing **MEETS COVERAGE CRITERIA**.

13) For individuals suspected of having malaria (see Note 10), the use of IFA for *Plasmodium* antibodies **DOES NOT MEET COVERAGE CRITERIA**.

14) For individuals suspected of having a rickettsial disease (see Note 11), the use of an IFA assay for IgG antibodies (two tests occurring a minimum of two weeks apart) **MEETS COVERAGE CRITERIA**.

15) For individuals suspected of having a rickettsial disease (see Note 11), the use of standard blood culture, NAAT, **or** IFA assay for IgM antibodies **DOES NOT MEET COVERAGE CRITERIA**.

16) For individuals suspected of having West Nile virus (WNV) disease (see Note 12), the use of IFA for WNV-specific IgG or IgM antibodies in either serum or CSF and a confirmatory plaque reduction neutralization test for WNV **MEETS COVERAGE CRITERIA**.

17) To confirm a WNV infection in individuals who are immunocompromised, nucleic acid detection of WNV **MEETS COVERAGE CRITERIA**.

18) For immunocompetent individuals suspected of having WNV disease (see Note 12), the use of NAAT for WNV **DOES NOT MEET COVERAGE CRITERIA.**

19) For individuals suspected of having a yellow fever virus (YFV) infection (see Note 13), the use of NAAT for YFV or serologic assays to detect virus-specific IgM and IgG antibodies, as well as a confirmatory plaque reduction neutralization test for YFV, **MEETS COVERAGE CRITERIA.**

20) For the detection of Zika virus, the use of NAAT **MEETS COVERAGE CRITERIA** in the following individuals:

    a) Up to 12 weeks after the onset of symptoms for symptomatic (see Note 8) pregnant individuals who, during pregnancy, have **either** lived in or traveled to areas with current or past Zika transmission **or** who have had sex with someone who either lives in or has recently traveled to areas with current or past Zika virus transmission (see Note 14).

    b) For symptomatic non-pregnant individuals living in or with recent travel to an area with an active CDC Zika Travel Health Notice or an area with current or past Zika virus transmission (see Note 14) when symptoms presented within the last seven days.

21) Zika virus NAAT and Zika virus IgM testing, as well as a confirmatory plaque reduction neutralization test for Zika, **MEETS COVERAGE CRITERIA** in **any** of the following situations:

   a) Up to 12 weeks after the onset of symptoms for symptomatic (see Note 8) pregnant individuals who, during pregnancy, have **either** lived in or traveled to areas with an active CDC Zika Travel Health Notice **or** who have had sex with someone who either lives in or has recently traveled to areas with an active CDC Zika Travel Health Notice (see Note 14).

   b) For pregnant individuals who have a fetus with prenatal ultrasound findings consistent with congenital Zika virus infection (see Note 15).

   c) For infants born from individuals who, during pregnancy, tested positive for Zika virus.

   d) For infants born with signs and symptoms of congenital Zika syndrome (see Note 15) and who have a birthing parent who had a possible Zika virus exposure during pregnancy.

   e) For symptomatic non-pregnant individuals living in or with recent travel to an area with an active CDC Zika Travel Health Notice or an area with current or past Zika virus transmission (see Note 14) when symptoms presented more than seven days prior to testing.

22) For non-pregnant individuals who have not traveled outside of the United States and its territories and who are symptomatic for Zika virus infection (see Note 8), NAAT and/or IgM testing for Zika detection **DOES NOT MEET COVERAGE CRITERIA**.

23) For asymptomatic individuals, testing for babesiosis, chikungunya virus, CTF, DENV, ehrlichiosis and/or anaplasmosis, malaria, rickettsial disease, TBRF, WNV, YFV, or Zika virus during a general exam without abnormal findings **DOES NOT MEET COVERAGE CRITERIA**.

NOTES:

Note 1: Typical signs and symptoms of babesiosis can include hemolytic anemia, splenomegaly, hepatomegaly, jaundice, and nonspecific flu-like symptoms such as fever, chills, body aches, weakness, and fatigue (CDC, 2024j).

Note 2: Typical signs and symptoms of HTRF (caused by *Borrelia miyamotoi*) can include chills or shakes, fatigue, nausea or vomiting, headache, and muscle and joint aches (CDC, 2024a).

Note 3: Typical signs and symptoms of LBRF (caused by *Borrelia recurrentis*) can include fever, headache, chills or shakes, muscle and joint aches, and nausea. Though the clinical symptoms of LBRF are similar to STRF, LBRF is usually associated with fewer relapses (CDC, 2024b)

Note 4: Typical signs and symptoms of STRF/TBRF (caused by *Borrelia hermsii*, *B. turicatae,* and other *Borrelia* bacteria) can include fever, headache, muscle aches, chills, dizziness, joint pain, nausea and vomiting, appetite loss, and rarely, facial paralysis eye pain or redness, or vision changes (CDC, 2024c).

Note 5: Typical signs and symptoms of chikungunya include high fever (>102◦F or 39◦C), joint pains (usually multiple joints, bilateral, and symmetric), headache, myalgia, arthritis, conjunctivitis, nausea, vomiting, and maculopapular rash (Staples et al., 2024).

Note 6: Typical signs and symptoms of CTF can include fever, chills, headache, myalgia, malaise, sore throat, vomiting, abdominal pain, and maculopapular or petechial rash (CDC, 2024e).

Note 7: Typical signs and symptoms of dengue include fever, headache, retro-orbital eye pain, myalgia, arthralgia, macular or maculopapular rash, petechiae, ecchymosis, purpura, epistaxis, gingival bleeding, hematuria, leukopenia, thrombocytopenia, hyponatremia, elevated AST and ALT, and nausea and/or vomiting (CDC, 2024f, 2024r).

Note 8: Typical signs and symptoms of Zika virus infection can include fever, rash, headache, joint pain, conjunctivitis (red eyes), and muscle pain (CDC, 2024t).

Note 9: Typical signs and symptoms of ehrlichiosis and/or anaplasmosis usually begin 5-14 days after an infected tick bite, and they include fever, headache, malaise, myalgia, and shaking chills. Ehrlichiosis can also present with gastrointestinal issues, including nausea, vomiting, and diarrhea (Biggs et al., 2016).

Note 10: Typical signs and symptoms of malaria can include fever, influenza-like symptoms (e.g., chills, headache, body aches), anemia, jaundice, seizures, mental confusion, kidney failure, and acute respiratory distress syndrome (Tan & Abanyie, 2024).

Note 11: Typical signs and symptoms of rickettsial diseases (including Rocky Mountain spotted fever, *Rickettsia parkeri* rickettsiosis, *Rickettsia* species 364D rickettsiosis, *Rickettsia* spp. (mild spotted fever), and *R. akari* (rickettsialpox)) usually begin 3 – 12 days after initial bite and can include fever, headache, chills, malaise, myalgia, nausea, vomiting, abdominal pain, photophobia, anorexia, and skin rash. *Rickettsia* species 364d rickettsiosis can also present with an ulcerative lesion with regional lymphadenopathy (Biggs et al., 2016).

Note 12: Typical signs and symptoms of WNV include headache, myalgia, arthralgia, gastrointestinal symptoms, and maculopapular rash. Less than 1% of infected individuals develop neuroinvasive WNV with symptoms of meningitis, encephalitis, or acute flaccid paralysis (Nasci et al., 2013).

Note 13: Typical signs and symptoms of yellow fever include symptoms of the toxic form of the disease (jaundice, hemorrhagic symptoms, and multisystem organ failure), as well as nonspecific influenza symptoms (fever, chills, headache, backache, myalgia, prostration, nausea, and vomiting in initial illness) (Gershman & Staples, 2024).

Note 14: The CDC provides information on the geographic risk classifications of Zika (https://www.cdc.gov/zika/geo/index.html), as well as providing travel health notices for pathogens of concern (https://wwwnc.cdc.gov/travel/notices).

Note 15: Typical signs and symptoms of congenital Zika syndrome can include microcephaly, problems with brain development, feeding problems (e.g., difficulty swallowing), hearing loss, seizures, vision problems, decreased joint movement (i.e., contractures), and stiff muscles (making it difficult to move) (CDC, 2024n).

Table of Terminology

Term	Definition
AAP	American Academy of Pediatrics
ASM	American Society for Microbiology
CDC	Centers for Disease Control and Prevention

CMS	Centers for Medicare and Medicaid Services
CSF	Cerebrospinal fluid
CTF/CTFV	Colorado tick fever /virus
CV	Coefficient of variation
DENV	Dengue virus
DENV NS1	Dengue virus nonstructural protein 1
DHF	Dengue hemorrhagic fever
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
EIA	Enzyme immunoassay
ELISA	Enzyme-linked immunosorbent assays
EM	Erythema migrans
ESR	Erythrocyte sedimentation rate
FDA	Food and Drug Administration
FFPE	Formalin-fixed, paraffin-embedded
FISH	Fluorescent in situ hybridization
GlpQ	Glycerophosphodiester phosphodiesterase gene
HAI	Hemagglutination inhibition test
HTRF	Hard tick relapsing fever
IDSA	Infectious Diseases Society of America
IEC	International Encephalitis Consortium
IFA	Indirect immunofluorescence antibody
IFAs	Immunofluorescence assays
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IHC	Immunohistochemistry
IMCA	Immunochemiluminometric assay
LBRF	Louse-borne relapsing fever
LDTs	Laboratory developed tests
MAbs	Monoclonal antibodies
MAC-ELISA	IgM antibody capture enzyme-linked immunosorbent assay
MIA	Microsphere-based immunoassay
MIF	Microimmunofluorescent
NAAT	Nucleic acid amplification testing
NDPH	New daily persistent headache
NNDSS	National Notifiable Disease Surveillance System
PCR	Polymerase chain reaction
PRNT	Plaque reduction neutralization test
PRNTs	Plaque reduction neutralization tests
PT	Prothrombin time
PTT	Partial thromboplastin time
qPCR	Quantitative polymerase chain reaction
RDT	Rapid diagnostic testing
RMSF	Rocky Mountain spotted fever
RNA	Ribonucleic acid
RT-PCR	Real-time polymerase chain reaction

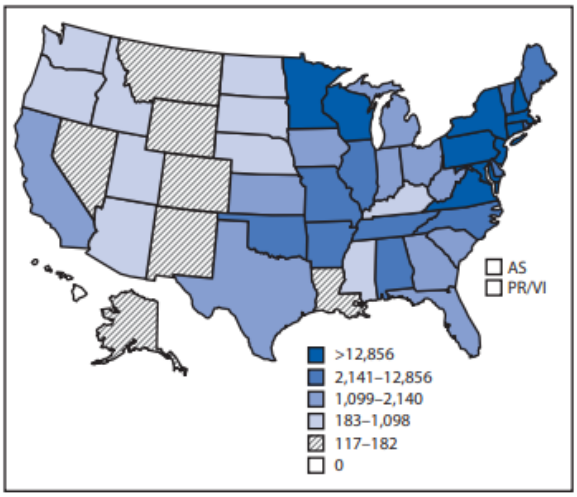
SFG	Spotted fever group
STRF	Soft tick relapsing fever
TBRF	Tickborne relapsing fever
WHO	World Health Organization
WNV	West Nile virus
YFV	Yellow fever virus

Scientific Background

Hematophagous arthropods, such as mosquitoes, ticks, fleas, and mites, can spread opportunistic bacteria, protozoa, and viruses to host organisms when feeding. Numerous outbreaks of arthropod-borne disease have been documented, including plague, an acute febrile disease caused by *Yersinia pestis* through the bite of infected fleas, which resulted in more than 50 million deaths in Europe alone during the "Black Death" outbreak. More than 3000 cases of plague were reported to the World Health Organization (WHO) between 2010 and 2015 with 584 deaths. Today, most cases of plague occur in the Democratic Republic of Congo, Madagascar, and Peru (WHO, 2022b).

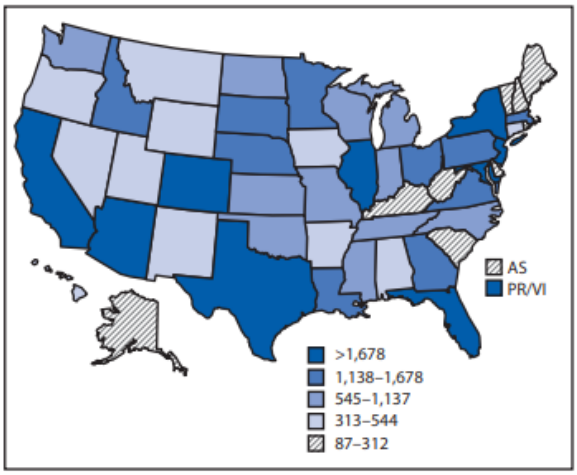
The Centers for Disease Control and Prevention (CDC) reported a large increase in the number of vector-borne diseases within the United States and its territories between 2004-2016. More than 640,000 cases were reported during that time; in fact, infections of tickborne bacteria and protozoa more than doubled from 2004 to 2016. "In the United States, 16 vector-borne diseases are reportable to state and territorial health departments, which are encouraged to report them to the National Notifiable Disease Surveillance System (NNDSS). Among the diseases on the list that are caused by indigenous pathogens are Lyme disease (*Borrelia burgdorferi*); West Nile, dengue, and Zika virus diseases; plague (*Yersinia pestis*); and spotted fever rickettsioses (e.g., *Rickettsia rickettsii*). Malaria and yellow fever are no longer transmitted in the United States but have the potential to be reintroduced" (Rosenberg et al., 2018). New vector-borne infections are emerging; for example, two unknown, life-threatening RNA viruses spread by ticks have been identified in the U.S. since 2004. Although both tick- and mosquito-borne diseases are increasing across the U.S., the CDC reports that these two vectors are showing different trends. The mosquito-borne diseases are characterized by epidemics; for example, West Nile Virus is essentially limited to the continental U.S. but has spread rapidly since its introduction to New York in 1999, whereas chikungunya and dengue primarily occur within the U.S. territories. On the other hand, the tickborne disease increase occurs in the continental U.S. and has experienced a gradual, steady rate increase with Lyme disease comprising 82% of all tickborne diseases (Rosenberg et al., 2018). Figure 1 and 2 below, taken from Rosenberg et al. (2018), show the reported cases of tickborne and mosquito-borne disease in the United States from 2004-2016.

FIGURE 1. Reported cases* of tickborne disease — U.S. states and territories, 2004–2016



Sources: CDC, National Notifiable Diseases Surveillance System, 2016 Annual Tables of Infectious Disease Data. https://wwwn.cdc.gov/nndss/infectious-tables.html. CDC, Division of Health Informatics and Surveillance. CDC, ArboNET.
Abbreviations: AS = American Samoa; PR/VI = Puerto Rico/U.S. Virgin Islands.
* Data classified by quintile.

FIGURE 2. Reported cases* of mosquitoborne disease — U.S. states and territories, 2004–2016



Sources: CDC, National Notifiable Diseases Surveillance System, 2016 Annual Tables of Infectious Disease Data. https://wwwn.cdc.gov/nndss/infectious-tables.html. CDC, Division of Health Informatics and Surveillance. CDC, ArboNET.
Abbreviations: AS = American Samoa; PR/VI = Puerto Rico/U.S. Virgin Islands.
* Data classified by quintile.

Rickettsial infections

Rocky Mountain spotted fever (RMSF) is the most common rickettsial infection in the U.S. with 6,248 cases reported to the CDC alone in 2017 (CDC, 2024q). RMSF is caused by *Rickettsia rickettsii*, spread in the U.S. predominantly by *Dermacentor variabilis* (the American dog tick*)* and *D. andersoni* (the Rocky Mountain wood tick), and can be found throughout North America as well as parts of South America. The Council for State and Territorial Epidemiologists combined RMSF with other rickettsial diseases into the more broad "spotted fever rickettsiosis" designation in 2010 (CDC, 2024q). Besides the obligatory tick bite, typical symptoms of RMSF include fever, headache, and rash with the characteristic rash occurring in approximately 88% to 90% of patients within three to five days of illness. If left untreated, RMSF can be fatal but can easily be treated with antimicrobial therapy upon timely diagnosis. Definitive diagnosis of RMSF cannot usually be made via culture because *Rickettsia* cannot be grown in cell-free culture media; they are obligate intracellular bacteria requiring living host cells. RMSF diagnosis can be made via either skin biopsy prior to treatment with antibiotics or through serologic testing using IFAs. Immunoglobulin G (Biggs et al.) antibodies are more specific than immunoglobulin M (IgM) antibodies since the latter can give false-positive results due to cross-reactivity with other bacterial pathogens. A drawback of IFA is that usually it is unreliable for the first five days of infection until antibody levels are high enough for detection. The CDC and major clinical labs do offer a polymerase chain reaction (PCR)-based assay for RMSF (McClain, 2024a).

Since 2001, thirteen more human *Rickettsiae* belonging to the spotted fever group (SFG) have been identified. All SFGs can cause fever, headache, and myalgia and are arthropod-borne (primarily ticks and mites). Most patients with an SFG display a rash and/or a localized eschar. Rickettsialpox, caused by *R. akari*, is transmitted from the bite of a house mouse mite, usually after mouse extermination programs result in a decrease of the mite's food supply. Rickettsialpox is typically a relatively mild disease that can resolve itself without treatment within three weeks, but treatment hastens improvement. Rickettsiosis can also be due to infection with *R. parkeri*, *R. amblyommii*, and *Rickettsia* species 364D (also called *R. philipii*). Isolation of SFG *Rickettsiae* is rare in clinical practice due to the difficulty of obtaining culture; consequently, serology, immunologic detection from tissue, and PCR are more often used for diagnosis.

Microimmunofluorescent (MIF) antibody tests, enzyme-linked immunosorbent assays (ELISAs), and Western blot immunoassays can be used to detect convalescent IgG and IgM antibodies, but these methods can only be used at least 10-14 days after the onset of illness when antibody concentrations are high enough for detection. McQuiston et al. (2014) concluded that the "use of IgM antibodies should be reconsidered as a basis for diagnosis and public health reporting of RMSF and other spotted fever group rickettsia in the United States" in one small study; the study demonstrated that IgM findings often resulted in false positives for Rock Mountain Spotted Fever and questioned the value of IgM testing (McQuiston et al., 2014). PCR is a very specific technique. PCR using tissue samples has higher specificity than whole blood PCR. Immunologic detection from a tissue biopsy requires the use of special laboratory equipment so it is not as frequently used as either the serologic or PCR detection methods (McClain, 2024c).

Ehrlichiosis and Anaplasmosis

Human ehrlichiosis was first reported in 1986, and the causative agent for human granulocytic anaplasmosis, *Anaplasma phagocytophilum*, was identified in 1994. Both ehrlichiosis and anaplasmosis are transmitted from the bite of infected ticks and have similar clinical and laboratory manifestations. Ehrlichiosis can be caused by *Ehrlichia chaffeensis, E. ewingii, and E. muris.* Typically, patients have a fever within an incubation period of one to two weeks. Other symptoms can include malaise, myalgia, headache, chills, gastrointestinal distress, and cough. Both leukopenia and thrombocytopenia can occur. Diagnosis via culture is extremely difficult. "Until 1995, only two isolates of *E. chaffeensis* had been recovered from humans; in both cases, this process required over 30 days of cultivation. The isolation of *A. phagocytophilum* from three additional patients has been accomplished using a cell culture system derived from human promyelocytic leukemia cells (McClain, 2024b). IFA testing for bacteria-specific antibodies is the most common method for diagnosing ehrlichiosis and anaplasmosis, but similar to rickettsiae, ELISA, PCR, and immunochemical tissue staining can be used as well. Unlike rickettsiosis, ehrlichiosis and anaplasmosis can also be detected by the presence of characteristic intraleukocytic morulae in a peripheral blood smear or buffy coat smear (McClain, 2024b).

Borrelia Infections

Besides Lyme disease, caused by *Borrelia burgdorferi*, *Borrelia* can cause relapsing fever. Tick-borne relapsing fever (TBRF) in North America is primarily caused by *B. hermsii*, *B. turicatae*, *B. parkeri, B. miyamotoi,* and *B. mazzottii*, and louse-borne relapsing fever (LBRF) is an infection caused by *B. recurrentis* (Barbour, 2024; Miller et al., 2024). The characteristic feature of these infections is the relapsing fever due to cyclical spirochetemia caused by antigenic variation of the spirochetes. Each bout of fever lasts three to 12 days with temperatures ranged from 39°C to 43°C (102.2°F to 109.4°F). Visual analysis by Giemsa or Wright staining blood smears taken during a febrile episode is common practice. PCR can also be used on a variety of samples, including cerebrospinal fluid (CSF), blood, tissue, or even culture medium. According to the CDC, "a change in serology results from negative to positive, or the development of an IgG response in the convalescent sample, is supportive of a TBRF diagnosis" (CDC, 2024p). One exception is using antibodies to the GlpQ protein characteristic of these *Borrelia* species but not to *B. burgdorferi* (Lyme disease) (Barbour, 2024).

Protozoa infections

Babesiosis is due to primarily *Babesia microti* in the U.S, but *B. divergens* and *B. venatorum* are the primary causative agents of babesiosis in Europe and China, respectively. The incubation period of *Babesia* depends on the mode of transfection: one to four weeks following a tick bite; the incubation

period after transfusion of contaminated blood products usually or three to seven weeks but ranges from one week to six months. The most common symptoms of infection include a fever, fatigue, malaise, chills, sweats, headache, and myalgia. Immunocompromised individuals can develop relapsing babesiosis due to an absent or impaired production of antibodies with approximately 20% mortality rate for patients who develop relapsing babesiosis. Most patients with babesiosis are also co-infected with other tickborne bacterial pathogens. "Preferred tools for diagnosis of babesiosis include blood smear for identification of Babesia organisms and polymerase chain reaction (PCR) for detection of Babesia DNA. Serology can be a useful adjunct to blood smear and PCR" (Krause & Vannier, 2024). Serology is not ideal in diagnosing an acute infection since antibody concentrations remain elevated post-recovery.

Plasmodium falciparum, P. vivax, and *P. ovale* are responsible for malaria. They are spread by the bite of an *Anopheles* mosquito where their sporozoites infect the liver within one to two hours. Within the hepatocyte, they form merozoites. Upon rupturing into the bloodstream, the merozoites infect red blood cells for trophozoite formation, causing the erythrocytic stage of the life-cycle where additional merozoites are released. During this stage of the cycle, the symptoms of malaria, including fever, occur. This process usually takes 12 to 35 days, but clinical manifestations can be delayed in individuals with partial immunity or those who are taking ineffective prophylaxis. Other initial symptoms can include irregular heartbeat, cough, anorexia, gastrointestinal distress, sweating, chills, malaise, arthralgia, and myalgia. Malaria, if left untreated, can also include acidosis, hypoglycemia, severe anemia, renal and hepatic impairment, edema, and death (Cohee & Seydel, 2022). Parasite-based diagnosis may include microscopic examination of blood smears, which can often identify the species of *Plasmodium* as well as the parasite density, and antigen-based tests. Rapid diagnostic testing (RDT) of the antigens using immunochromatographic methods is available, but the accuracy of the RDT can vary considerably. NAATs can also be used to identify a malarial infection, and NAATs "are typically used as a gold standard in efficacy studies for antimalarial drugs, vaccines, and evaluation of other diagnostic agents" with a "theoretical limit of detection for PCR…estimated at 0.02 to 1 parasite/microL" (Hopkins, 2023). The Mayo Clinic Laboratories indicates that "PCR is an alternative method of malaria diagnosis that allows for sensitive and specific detection of Plasmodium species DNA from peripheral blood. PCR may be more sensitive than conventional microscopy in very low parasitemias, and is more specific for species identification…Malaria PCR can be used in conjunction with traditional blood film or Babesia PCR when the clinical or morphologic differential includes both babesiosis and malaria" Clinic (2024).

Viral infections

Examples of arthropod-borne viruses (arboviruses) include West Nile virus (WNV), dengue, yellow fever virus (YFV), chikungunya, and Colorado tick fever virus. In the United States, WNV is the most common arbovirus reported to the CDC. In 2016, 96% of the reported 2,240 cases of domestic arboviruses were WNV with 61% of the WNV cases reported being neuroinvasive. Neuroinvasive WNV includes meningitis, encephalitis, and acute flaccid paralysis (Burakoff et al., 2018). In general, most infected individuals are asymptomatic with only 20-40% of infected patients showing any characteristic symptoms of WNV, including fever, headache, malaise, myalgia, anorexia, and rash. Diagnosis of WNV of a symptomatic individual usually occurs with a WNV IgM antibody capture ELISA (MAC-ELISA) assay. A patient with symptoms of a neurologic infection does require a lumbar puncture. Confirmatory testing can include a plaque reduction neutralization test (PRNT). PCR testing is primarily used with immunocompromised patients who have delayed or absent antibody production, patients with a history of prior flavivirus infections, and blood donors who may be asymptomatic (Petersen, 2022).

Dengue virus (DENV) infection is a result of being bitten by an infected *Aedes aegypti* or *A. albopictus* mosquito. Four distinct DENV types of *Flavivirus* are known: DENV-1, DENV-2, DENV-3, and DENV-4.

DENV is endemic throughout much of the tropical regions of the world, but the only region of the U.S. endemic for DENV is Puerto Rico. The last major outbreak occurred in Puerto Rico in 2010 where 26,766 cases of suspected DENV were reported and 47% of all laboratory tested specimen were positive (CDC, 2024f). "Dengue fever…is an acute febrile illness defined by the presence of fever and two or more of the following but not meeting the case definition of dengue hemorrhagic fever: headache, retro-orbital or ocular pain, myalgia and/or bone pain, arthralgia, rash, hemorrhagic manifestations…[and] leukopenia. The cardinal feature of dengue hemorrhagic fever is plasma leakage due to increased vascular permeability as evidenced by hemoconcentration (≥20 percent rise in hematocrit above baseline), pleural effusion, or ascites. DHF [dengue hemorrhagic fever] is also characterized by fever, thrombocytopenia, and hemorrhagic manifestations…." (Thomas et al., 2022). Laboratory diagnostic testing includes direct detection of viral components in serum or indirect serologic assays. "Detection of viral nucleic acid or viral antigen has high specificity but is more labor intensive and costly; serology has lower specificity but is more accessible and less costly" (Thomas et al., 2022). Culture testing as a diagnostic tool usually is time-prohibitive.

Zika virus is a mosquito-borne illness discovered in Uganda in 1947 but has since spread across Asia and to the Americas. Zika infection has been tied to several birth defects. The first human cases of Zika were detected in 1952. Prior to 2007, at least 14 cases of Zika had been documented. Symptoms of Zika are similar to those of many other diseases; therefore, many cases may not have been recognized (CDC, 2024t). The most common symptoms of Zika are fever, rash, joint pain, and conjunctivitis (CDC, 2024t). The illness is usually mild with symptoms beginning two to seven days after being bitten by an infected mosquito, lasting for several days to a week. Most individuals infected with Zika virus are unaware of the infection, as only a maximum of 25% of people infected will exhibit symptoms (CDC, 2024t; LeBeaud, 2023). Diagnosis of the Zika virus is definitively established through reverse-transcription polymerase chain reaction (RT-PCR) for Zika virus RNA in all symptomatic patients. Aside from pregnant individuals who have traveled to an at risk area, asymptomatic patients are typically not tested (LeBeaud, 2023).

Colorado tick fever virus (CTFV) is a *Reoviridae* transmitted primarily by the Rocky Mountain wood tick (*Dermacentor andersoni*) in the western U.S. and Canada. Transmission of CTFV has also been reported in blood transfusions. The incubation period can last up to 14 days, and symptoms include fever, headache, chills, myalgia, leukopenia, and prostration. Only 15% of symptomatic patients demonstrate a rash. Serologic tests are usually not helpful until at least 10-14 days for antibody production whereas real-time PCR (RT-PCR) can be used on the first day of symptoms (Petersen, 2021).

Yellow fever, occurring primarily in sub-Saharan Africa and South America, is a flavivirus spread by mosquitoes that causes hemorrhagic fever with a high fatality rate. An outbreak in Brazil in January-March 2018 resulted in four of ten patients infected with YFV dying. None of those showing symptoms had been vaccinated against YFV. Yellow fever causes hemorrhagic diathesis due to decreased synthesis of vitamin K-dependent coagulation factors as well as hepatic dysfunction, renal failure, and coagulopathy. Yellow fever diagnosis is typically made by a serologic test using an ELISA-IgM assay; however, this assay does cross-react with other flaviviruses and with the YFV vaccination. Rapid diagnostic testing using either PCR or immunoassay is available. Viral isolation and culture can be performed, but it requires inoculation of mosquitoes or mammalian cell culture. Tissue biopsy, such as liver, cannot be performed on the living patient due to possible fatal hemorrhaging; biopsy would be performed during the post-mortem workup (Wilder-Smith, 2024).

Chikungunya virus, endemic in many tropical and subtropical regions of the world, is transmitted by the mosquitoes *Aedes aegypti* and *Aedes albopictus*. Within the U.S., chikungunya is prevalent in Puerto Rico where approximately 25% of blood donors were seropositive; it has also been reported in Florida. Both

dengue and Zika are transmitted by the same vectors, so these viruses often co-circulate geographically Chikungunya can cause acute febrile polyarthralgia and arthritis. The predominant testing method for diagnosis of chikungunya is the detection of viral RNA via either RT-PCR or virus serology using either ELISA or IFA. Viral culture is typically not used as a diagnostic tool but is used for epidemiologic research (Wilson & Lenschow, 2022).

Types of Testing

Test	Description	Rationale
Culture	Culture growth depends on the pathogen being studied. If the pathogen is an obligate intracellular organism, then it must be isolated using more sophisticated cell culture techniques. In many circumstances, culture is used for research and/or epidemiology rather than as a diagnostic tool (Biggs et al., 2016; Miller et al., 2024).	At times, culture testing is not as sensitive as either NAAT or serologic testing and can be time-intensive when treatment should not be delayed. Depending on the organism, this may require high biosafety level laboratory for culture growth (Biggs et al., 2016).
Indirect immunofluorescene antibody (IFA) assays	IFA is a serologic assay that can be used to test for the presence of antibodies, such as IgG and IgM, reactive against the pathogen (Biggs et al., 2016).	Depending on the pathogen, IFA can be a useful tool. At times, though, it can cross-react with either a prior vaccination or infection (Wilder-Smith, 2024). An acute infection can often be determined by performing IFA in both the acute phase and convalescent phase where at least a fourfold increase in antibodies is indicative of an acute infection (Biggs et al., 2016).
Darkfield microscopy	Darkfield microscopy can be used to detect the presence of microorganisms, such as motile spirochetes (Miller et al., 2024).	This technique is not widely available, and transport of sample must be done immediately if testing of motile specimen is desired (Miller et al., 2024).
Blood-smear microscopy	Blood-smear microscopy can be either thick or thin and is typically performed on a sample stained with an eosin-azure-type dye, such as Giemsa, to look at intracellular structures or morphological features (Biggs et al., 2016).	This technique should be performed by an experienced microscopist since it can be inconsistent. As compared to other techniques, this technique is relatively inexpensive (Biggs et al., 2016).
Nucleic acid amplification testing (NAAT)	NAATs can include polymerase chain reaction (PCR), real-time PCR (RT-PCR), or other enzyme-dependent amplification testing for the presence of nucleic acids (DNA or RNA).	NAATs can be specific and sensitive; however, they may not be available at all laboratories and/or can be costly. Some NAATs are available as rapid diagnostic tools. NAATs have

		been used on serum, whole blood, tissue, CSF, and even formalin-fixed, paraffin-embedded biopsies from autopsy tissues. The sensitivity of the technique can vary depending on the sample; for example, whole blood PCR for *R. rickettsii* is less sensitive than a similar sample test for *E. chaffeensis* (Biggs et al., 2016).

Analytical Validity

The use of antibodies to detect and diagnose arthropod-associated infections and diseases is a common practice. Johnson et al. (2000) first reported the use of monoclonal antibody-based capture ELISA testing for a variety of alphaviruses, including chikungunya, flaviviruses, including dengue and yellow fever, and bunyaviruses. The researchers concluded, "IgG ELISA results correlated with those of the standard plaque-reduction neutralization assays. As expected, some test cross-reactivity was encountered within the individual genera, and tests were interpreted within the context of these reactions. The tests were standardized for laboratory diagnosis of arboviral infections, with the intent that they be used in tandem with the corresponding IgM antibody-capture ELISAs" (Johnson et al., 2000). Kalish et al. (2001) also demonstrated that IgG and/or IgM antibody responses can still occur up to 20 years post-infection; consequently, a rise in antibody titer does not necessarily indicate a current, acute infection (Kalish et al., 2001).

Granger and Theel (2019) published an evaluation of two enzyme-linked immunosorbent assays and a rapid immunochromatographic assay for the detection of IgM antibodies to Zika virus. This article states that five serological assays have been approved by the FDA in an emergency use situation and include the Chembio DPP Zika IgM system (a rapid immunochromatographic assay), the InBios ZIKV Detect 2.0 IgM antibody capture enzyme-linked immunosorbent assay, and the InBios ZIKV Detect MAC-ELISA. These three serologic assays were evaluated, using 72 samples, based on the identification of neutralizing antibodies to Zika virus, dengue virus, or West Nile virus. "The Chembio DPP Zika ICA and InBios ZIKV 2.0 MAC-ELISA showed 95% specificity in 22 ZIKV/DENV-seronegative specimens and in 13 samples positive for NAbs to non-ZIKV flaviviruses. Comparatively, the InBios ZIKV MAC-ELISA was "presumptive" or "possible Zika positive" in 8 of 12 WNV or DENV PRNT-positive samples and in 12 of 22 PRNT-seronegative sera" (Granger & Theel, 2019). The authors conclude that by replacing the InBios ZIKV MAC-ELISA with the InBios ZIKV 2.0 MAC-ELISA, testing burden will be minimized on laboratories performing PRNT for the identification of neutralizing antibodies.

Leski et al. (2020) performed a 2020 study published in the *Malaria Journal* that compared traditional diagnostic methods such as rapid diagnostic tests (RDTs) and DNA-based methods to polymerase chain reaction (PCR). The results indicated consistency with "previous observations that PCR-based tests have a significantly higher sensitivity when compared with both microscopy and RDTs" (Leski et al., 2020).

Mathison and Pritt (2017) reviewed current standards for malaria testing and the most used methods for laboratory diagnosis. The most common tests "are microscopic examination of stained blood films and detection of parasite antigen or nucleic acid… Rapid antigen detection methods and molecular amplification tests are also increasingly employed for malaria diagnosis and are useful adjunctive tests."

According to the algorithm developed in "Update on Malaria Diagnostics and Test Utilization," NAAT tests are one of three tests recommended for use if malaria is suspected based on clinical findings and exposure history (Mathison & Pritt, 2017).

Kim et al. (2018) had also developed a rapid diagnostic test (RDT) for detecting IgG/IgM antibodies against Zika virus using "monoclonal antibodies to the envelope (E) and non-structural protein (NS1)." The diagnostic accuracy of this kit was "fairly high; sensitivity and specificity for IgG was 99.0 and 99.3%, respectively, while for IgM it was 96.7 and 98.7%, respectively." However, there were cross reactions with the dengue virus evaluated using anti-Dengue Mixed Titer Performance Panel (PVD201), "in which the Zika RDT showed cross-reactions with [dengue virus] in 16.7% and 5.6% in IgG and IgM, respectively." This research could potentially enable the rapid diagnostic test to be preferable to the traditional RT-PCR in endemic areas (Kim et al., 2018).

Clinical Utility and Validity

Kato et al. (2013) tested the sensitivity of two different RT-PCR-based assays for Rickettsia—PanR8, an assay that tests for Rickettsia in general, and RRi6, an assay specific for *R. rickettsii*. Both of these methods were more sensitive in testing for Rickettsia than the nested PCR method of the CDC; moreover, both of these methods are faster than the nested PCR method (one hour versus one to two days, respectively) (Kato et al., 2013). These results were corroborated in 2014 by Denison and colleagues. They used a multiplex PCR assay to correctly identify all cell controls for *R. rickettsii*, *R. parkeri*, and *R. akari*; moreover, no false-positive results were reported using this methodology. "This multiplex real-time PCR demonstrates greater sensitivity than nested PCR assays in FFPE [formalin-fixed, paraffin-embedded] tissues and provides an effective method to specifically identify cases of Rocky Mountain spotted fever, rickettsialpox, and *R. parkeri* rickettsiosis by using skin biopsy specimens" (Denison et al., 2014).

The FDA has approved the use of the BinaxNOW malaria test for screening and diagnosing malaria. Even though this testing method is considerably faster than other methods (as low as 1.1-1.7 hours complete turnaround time (Ota-Sullivan & Blecker-Shelly, 2013), the use of BinaxNOW in non-endemic areas is a point of controversy due to relatively low sensitivity (84.2%) and for misclassifying Plasmodium falciparum malaria as non-falciparum (Dimaio et al., 2012). Moreover, it has been reported that Salmonella typhi can give a false-positive for malaria using the BinaxNOW test (Meatherall et al., 2014).

van Bergen et al. (2021) evaluated a novel real-time PCR assay for clinical validity. The authors used reference samples, patient samples, and synthetic controls. The analytical performance details of the MC004 assay were considered: "analytical specificity, limit of detection, the ability to detect mixed infections, and the potential to determine the level of parasitaemia of P. falciparum, including assessment of within-run and between-run precisions." The authors reported "zero false positive or false negative results." Regarding precision, "the within-run and between-run precisions were less than 20% CV at the tested parasitaemia levels of 0.09%, 0.16%, 2.15% and 27.27%." Based on these results, the authors reported that "the entry of PCR-based techniques into malaria diagnostics has improved the sensitivity and specificity of the detection of Plasmodium infections… Based upon the analytical performance characteristics that were determined, the MC004 assay showed performance suitable for use in clinical settings, as well as epidemiological studies" (van Bergen et al., 2021).

Akoolo et al. (2017) compared qPCR results in the detection of Babesia infection against currently available non-NAAT tests (FISH and microscopy). Blood samples were analyzed from 192 patients. The researchers report that "Of 28 samples that were positive by FISH, 27 (96%) were also positive by qPCR

indicating high congruency between nucleic acid-based tests. Interestingly, of 78 asymptomatic samples not tested by FISH, 22 were positive by our qPCR" (Akoolo et al., 2017). Overall, the qPCR method was found to have a sensitivity of 96.2% and a specificity of 70.5%. The authors conclude, "Robust qPCR using specific probes can be highly useful for efficient and appropriate diagnosis of babesiosis in patients in conjunction with conventional diagnostics, or as a stand-alone test, especially for donated blood screening" (Akoolo et al., 2017).

Reynolds et al. (2017) examined the 2016 United States Pregnancy Registry to estimate the proportion of birth defects of pregnant women exposed to Zika, and out of 972 pregnancies with laboratory evidence of a possible Zika infection, 51 had birth defects (five percent). Of the 250 confirmed infections, 24 had birth defects. Similarly, Shiu et al. (2018) evaluated the screening results of the Zika virus in Miami-Dade County in Florida. Of 2327 women screened for Zika, 86 had laboratory evidence of infection, and two had congenital Zika "syndrome" (Zika-caused birth defects) (Shiu et al., 2018).

Guidelines and Recommendations

Centers for Disease Control and Prevention (CDC)

Diagnosis and Management of Tickborne Rickettsial Diseases (Biggs et al., 2016): In 2016, the CDC released their guidelines and recommendations concerning Rickettsial diseases, including Rocky Mountain spotted fever, in the Morbidity and Mortality Weekly Report. The table below summarizes their recommended diagnostic tests for tickborne rickettsial diseases:

TABLE 4. Recommended diagnostic tests for tickborne rickettsial diseases

Disease	PCR			Microscopy for morulae detection	IFA assay for IgG antibodies (acute and convalescent)*
	Whole blood	Eschar biopsy or swab	Rash biopsy		
Rocky Mountain spotted fever	Yes†	—	Yes	—	Yes
Rickettsia parkeri rickettsiosis	—	Yes	Yes	—	Yes
Rickettsia species 364D rickettsiosis	—	Yes	—	—	Yes
Ehrlichia chaffeensis ehrlichiosis (human monocytic ehrlichiosis)	Yes	—	—	Yes	Yes
Ehrlichia ewingii ehrlichiosis	Yes	—	—	Yes	Yes
Ehrlichia muris-like agent ehrlichiosis	Yes	—	—	—	Yes
Human anaplasmosis (human granulocytic anaplasmosis)	Yes	—	—	Yes	Yes

Abbreviations: IFA = indirect immunofluorescence antibody; IgG = immunoglobulin G; PCR = polymerase chain reaction.
* IFA assay is insensitive during the first week of illness for most tickborne rickettsial diseases; a sample should be collected during this interval (acute specimen), and a second sample should be collected 2–4 weeks later (convalescent specimen) for comparison. Elevated titers alone are not sufficient to diagnose infection with tickborne rickettsial diseases; serial titers are needed for confirmation. Demonstration of at least a fourfold rise in antibody titer is considered confirmatory evidence of acute infection.
† PCR of whole blood samples for *Rickettsia rickettsii* has low sensitivity; sensitivity increases in patients with severe disease.

To summarize their recommendations, even though indirect immunofluorescence antibody assays (IFAs) are insensitive typically during the first week of an acute infection, they are the standard reference for tickborne rickettsial infections; in addition, a minimum of two tests are to be performed for a diagnosis. Usually, one sample is taken early after the initial symptoms are present, and a second sample is taken two to four weeks later. A minimum of a fourfold rise in antibody titer is required to confirm diagnosis. In cases of ehrlichiosis and anaplasmosis, during the first week, PCR amplification can be used on whole blood for diagnosis, but PCR has low sensitivity in Rocky Mountain spotted fever except in patients with severe disease. Morulae detection via either blood smear or buffy coat preparation microscopy can also be indicative of ehrlichiosis or anaplasmosis. However, "Rickettsiae cannot be isolated with standard blood culture techniques because they are obligate intracellular pathogens; specialized cell culture methods are required. Because of limitations in availability and facilities, culture is not often used as a routine confirmatory diagnostic method for tickborne rickettsial diseases" (Biggs et al., 2016).

In 2024, the CDC published updated guidelines pertaining to rickettsial infections, which provide similar guidelines to those published in 2016. "The standard serologic test for diagnosis of RMSF is the indirect fluorescent antibody (IFA) test for immunoglobulin G (IgG) using *R. rickettsii* antigen. IgG IFA assays should be performed on paired acute and convalescent serum samples collected 2–10 weeks apart to demonstrate evidence of a fourfold seroconversion. Single or inappropriately timed serologic tests, in relation to clinical illness, can lead to misinterpretation of results" (CDC, 2024d). They also provide statements on nucleic acid testing and IHC/culture testing for rickettsial infections: "PCR amplification is performed on DNA extracted from whole blood serum, or plasma. *R. rickettsii* infect the endothelial cells that line blood vessels and may not circulate in large numbers in the blood until the disease has progressed to a severe phase of infection. Although a positive PCR result is helpful, a negative result does not rule out the diagnosis, and treatment should not be withheld due to a negative result. PCR might also be used to amplify DNA from a skin biopsy of a rash lesion, or in post-mortem tissue specimens. . . Culture and IHC assays can also be performed on skin biopsies of a rash lesion, or post-mortem tissue specimens. Culture isolation and IHC assays of *R. rickettsii* are only available at specialized laboratories; routine hospital blood cultures cannot detect the organism" (CDC, 2024d).

Soft tick relapsing fever (STRF) /Tickborne relapsing fever (TBRF) (CDC, 2024c, 2024i): In the U.S., STRF/TBRF can be caused by *Borrelia hermsii, B. turicatae,* and other *Borrelia* bacteria via the bite of soft-bodied *Ornithodoros* genus ticks. STRF often presents with a relapsing nature, with symptoms appearing 4-21 days after exposure, with intermittent fevers lasting for three days and remitting for seven days before relapse. Moreover, "Spirochetes may be present in high concentrations in the blood of febrile patients (>10^6 spirochetes/ml). Spirochetes are most readily detected by microscopy in symptomatic, untreated patients early in the course of infection. Direct visualization by microscopy using dark field or stained peripheral blood smears is generally adequate to confirm the diagnosis… PCR is more sensitive than microscopy and may also be used during asymptomatic periods or soon after treatment initiation. The preferred specimen type for PCR testing is whole blood… Serologic testing is available from some labs to diagnose STRF. Serologic assay results are most sensitive when specimens are collected at least 14 days after symptom onset… Patients with relapsing fevers might have false positive serologic tests for Lyme disease" (CDC, 2024c).

The CDC acknowledges that some PCR and serologic tests may cross-react with other *Borrelia* species; thus, "clinical and epidemiologic features, such as travel and exposure history, are important to guide interpretation of test results. Consider a diagnosis of STRF for patients with positive Lyme disease or [hard tickborne relapsing fever] serology who have not been in areas endemic for these diseases." Additionally, patients may exhibit other general laboratory findings, such as "thrombocytopenia, increased white blood cell count, mildly increased serum bilirubin level, elevated erythrocyte sedimentation rate (ESR), and slightly prolonged prothrombin time (PT) and partial thromboplastin time (PTT) (CDC, 2024i).

Hard tick relapsing fever (HTRF) (CDC, 2024a, 2024g): In the U.S., HTRF is used to differentiate between infections caused by hard-bodied ticks and soft-bodied ticks (see STRF above). HTRF is caused by the *Borrelia miyamotoi* bacteria and is transmitted through the bites of infected blacklegged ticks (*Ixodes scapularis*) and western blacklegged ticks *(Ixodes pacificus*). Unlike STRF, it causes a single episode of fever more commonly, with 10% of cases having a relapsing fever. Symptoms appear about two weeks after a tick bite but can occur within three to six days after exposure. Diagnosis is often made by PCR using whole blood, but several PCR and serologic methods cannot distinguish between HTRF and STRF. The CDC also adds "Serologic testing is available from some labs for diagnoses of HTRF. Serologic assay results are most sensitive when specimens are collected at least 14 days after symptom onset. Serum taken early during infection may yield negative results." Similar emphasis is placed on considering

clinical and epidemiological features when interpreting results, as HTRF patients may also test positive for other *Borrelia* species, such as Lyme disease (CDC, 2024a, 2024g).

Louse-borne relapsing fever (LBRF) (CDC, 2024b, 2024h): In the U.S., LBRF is caused by *Borrelia recurrentis* bacteria and transmitted by the human body louse, and rarely, head louse. It also occurs endemically in regions of Africa and in overcrowded conditions. Clinically, LBRF presents similarly to STRF but with fewer relapses. Diagnosis is made with "direct visualization of spirochetes in a peripheral blood smear in symptomatic, untreated patients early in the course of infection," as "people with LBRF experience high levels of spirochetemia during febrile episodes." Alternatives for diagnosis also include PCR, but the same precautions hold for LBRF as for HTRF and STRF when interpreting results (CDC, 2024b, 2024h).

Colorado Tick Fever (CTF) (CDC, 2024e): As of 2023, CTF was reportable in Arizona, Colorado, Idaho, Montana, New Mexico, Oregon, South Dakota, Utah, Washington, and Wyoming. "Laboratory diagnosis of CTF is generally accomplished by testing of serum to detect viral RNA or virus-specific immunoglobulin (Ig) M and neutralizing antibodies. Antibody production can be delayed with CTF, so tests that measure antibodies may not be positive for 14–21 days after the onset of symptoms. RT-PCR (reverse-transcriptase polymerase chain reaction) is a more sensitive test early in the course of disease. CTF testing is available at some commercial and state health department laboratories and at CDC. Contact your state or local health department for assistance with diagnostic testing. They can help you determine if samples should be sent to the CDC Arbovirus Diagnostic Laboratory for further testing" (CDC, 2024e).

Babesiosis (CDC, 2024j): Babesiosis is caused most commonly by *Babesia microti*, which is usually transmitted by white-footed mice and other small mammals. Diagnosis can be challenging due to the nonspecific clinical manifestations of the disease. "For acutely ill patients, the findings on routine laboratory testing frequently include hemolytic anemia and thrombocytopenia. Additional findings may include proteinuria, hemoglobinuria, and elevated levels of liver enzymes, blood urea nitrogen, and creatinine. When considering a babesiosis diagnosis, healthcare providers should explicitly request a manual (non-automated) review of the peripheral blood smear. In symptomatic patients with acute infection, it is typical to detect *Babesia* parasites through light-microscopic examination of blood smears, though multiple smears may need to be examined. Distinguishing between *Babesia* and *Plasmodium* (especially *P. falciparum*) parasites and artifacts like stain or platelet debris can be challenging. Consider having a reference laboratory confirm the diagnosis—by blood-smear examination and, if indicated, by other means, such as molecular and/or serologic methods tailored to the setting/species" (CDC, 2024j).

Malaria (Tan & Abanyie, 2024): The CDC considers smear microscopy as the gold standard in diagnosing malaria since it can determine the species, identify the stage of parasitic life-cycle, and quantify the parasitemia. The CDC states, "Blood smear microscopy remains the most important method for malaria diagnosis. Microscopy can provide immediate information about the presence of parasites, allow quantification of the density of the infection, and allow determination of the species of the malaria parasite—all of which are necessary for providing the most appropriate treatment. Tests should be performed immediately when ordered by a health care provider, and microscopy results should be available as soon as possible, ≤24 hours of the patient's presentation. They should not be saved for the most qualified staff to perform or batched for convenience. In addition, these tests should not be sent out to reference laboratories with results available only days to weeks later. Assistance with speciation of malaria on smears is available from CDC" (Tan & Abanyie, 2024). The CDC also notes that rapid diagnostic tests (RDTs) for malaria can detect malaria parasitic antigens. However, "RDTs offer a useful alternative to microscopy in situations where reliable microscopic diagnosis is not immediately available. Although RDTs can detect malaria antigens within minutes, they have several limitations. RDTs cannot

distinguish between all of the *Plasmodium* species that affect humans, they may be less sensitive than expert microscopy or PCR for diagnosis, they cannot quantify parasitemia, and an RDT-positive test result may persist for days or weeks after an infection has been treated and cleared. Thus, RDTs are not useful for assessing response to therapy. Furthermore, in some areas, mutations are increasingly being observed in malaria parasites, resulting in an absence of the malaria antigen usually detected by many RDTs, including the only RDT used in the United States. The absence of this parasite antigen in peripheral blood can lead to false-negative RDT test results. Both positive and negative RDT results must always be confirmed by microscopy. Microscopy confirmation of the RDT result should occur as soon as possible, because the information on the presence, density, and parasite species is critical for optimal management of malaria" (Tan & Abanyie, 2024). Regarding PCR, the CDC states that "These tests are more sensitive than routine microscopy, but results are not usually available as quickly as microscopy results, thus limiting the utility of this test for acute diagnosis and initial clinical management. Use of PCR testing is encouraged to confirm the species of malaria parasite and detect mixed infections" (Tan & Abanyie, 2024).

While diagnosis from microscopic examination remains the gold standard for laboratory confirmation of malaria, the CDC does acknowledge that antigen detection with a rapid diagnostic test and molecular diagnosis by PCR may be useful in certain situations: "In the international setting, various test kits are available to detect antigens derived from malaria parasites. Such immunologic ("immunochromatographic") tests most often use a dipstick or cassette format and provide results in 2-15 minutes. These "Rapid Diagnostic Tests" (RDTs) offer a useful alternative to microscopy in situations where reliable microscopic diagnosis is not available. Malaria RDTs are currently used in some clinical settings and programs. On June 13, 2007, the U.S. Food and Drug Administration (FDA) approved the first RDT for use in the United States. This RDT is approved for use by clinical laboratories, not by individual clinicians or by patients themselves. It is recommended that all RDTs are followed-up with microscopy to confirm the results and if positive, to confirm the species and quantify the proportion of red blood cells that are infected. The use of this RDT may decrease the amount of time that it takes to determine whether a patient is infected with malaria. . . Parasite nucleic acids are detected using polymerase chain reaction (PCR). Although this technique may be more sensitive than blood smear microscopy, it is of limited utility for the diagnosis of acutely ill patients in the standard healthcare setting. PCR results are often not available quickly enough to be of value in establishing the diagnosis of malaria infection. PCR is most useful for confirming the species of malarial parasite after the diagnosis has been established by either smear microscopy or RDT" (CDC, 2024k).

Chikungunya (Staples et al., 2024): In the CDC Yellow Book, concerning the Chikungunya virus, they recommend that "the differential diagnosis of chikungunya virus infection depends on clinical features (signs and symptoms) as well as where the person was suspected of being infected. Consider other diseases in the differential diagnosis, including adenovirus, other alphaviruses (Barmah Forest, Mayaro, O'nyong-nyong, Ross River, and Sindbis), dengue, enterovirus, leptospirosis, malaria, measles, parvovirus, rubella, group A *Streptococcus*, typhus, Zika, and postinfectious arthritis and rheumatologic conditions. Laboratory diagnosis is done by serum testing for detection of virus, viral nucleic acid, or virus-specific IgM and neutralizing antibodies. Because the virus develops high levels of viremia during the first week after symptom onset, chikungunya can often be diagnosed by performing viral culture or nucleic acid amplification on serum. Virus-specific IgM antibodies normally develop toward the end of the first week of illness but can remain detectable for months to years after infection. Rarely, serum IgM antibody testing can yield false-positive results due to cross-reacting antibodies against related alphaviruses (e.g., Mayaro virus, O'nyong-nyong virus)…Testing for chikungunya virus is performed at several state health department laboratories, and commercial laboratories" (Staples et al., 2024).

West Nile Virus (WNV) (CDC, 2024o): "The front-line screening assay for laboratory diagnosis of human WNV infection is the IgM assay. Currently, the FDA has cleared three commercially available test kits from different manufacturers, for detection of WNV IgM antibodies…In addition, the CDC-defined IgM and IgG EIA [i.e., ELISA or microsphere-based immunoassay (MIA)] can be used…The CDC MIA can differentiate WNV from St. Louis encephalitis…Because the IgM and IgG antibody tests can cross-react between flaviviruses (e.g., [St. Louis encephalitis], dengue, yellow fever, WNV, Powassan), they should be viewed as screening tests only. For a case to be considered confirmed, serum samples that are antibody-positive on initial screening should be evaluated by a more specific test; currently the plaque reduction neutralization test (PRNT) is the recommended test for differentiating between flavivirus infections… Specimens submitted for WNV testing should also be tested against other arboviruses known to be active or be present in the area or in the region where the patient traveled."

There are also virus detection assays that can be utilized to detect viable WNV, WNV antigen or WNV RNA in human samples, but they vary in sensitivity, specificity, and time required to conduct the test. However, the CDC warns that "viremia is almost always absent by the time a patient presents with neuroinvasive illness and thus viral isolation is generally not recommended as part of a testing algorithm in immune competent patients…Confirmation of virus isolate identity can be accomplished by indirect immunofluorescence assay (IFA) using virus-specific monoclonal antibodies (MAbs) or nucleic acid detection (e.g. RT-PCR, real-time RT-PCR or sequencing)… Virus isolation or RT-PCR on serum may be helpful in confirming WNV infection in immunocompromised patients when antibody development is delayed or absent" (CDC, 2024o).

Yellow Fever Virus (YFV) (Gershman & Staples, 2024): Isolation of the virus or NAAT should be performed as early as possible in suspected cases of YFV. "By the time more overt symptoms are recognized, the virus or viral RNA may no longer be detectable; thus, virus isolation and nucleic acid amplification should not be used to rule out a diagnosis of YF. Serologic assays can be used to detect virus-specific IgM and IgG antibodies. Because of the possibility of cross-reactivity between antibodies against other flaviviruses, however, more specific antibody testing (e.g., a plaque reduction neutralization test) should be performed to confirm the infection" (Gershman & Staples, 2024). Since YFV is a nationally notifiable disease, clinicians should contact their state and/or local health departments or call the CDC Arboviral Diseases Branch according to their respective local, state, and/or federal guidelines. As of May 2023, "Only one YF vaccine (YF-VAX, Sanofi Pasteur) is licensed for use in the United States. Periodically in the United States, shortages of YF-VAX have occurred due to production issues, including one that lasted from late 2015 until early 2021. To address this most recent shortage, Sanofi Pasteur collaborated with the CDC and the U.S. Food and Drug Administration (FDA) to import and distribute Stamaril (a YF vaccine comparable to YF-VAX, manufactured at the company's facility in France) under an expanded-access investigational new drug protocol" (Gershman & Staples, 2024).

Dengue (CDC, 2024m): Diagnosis of dengue can be diagnosed differently based on the phase: the acute phase (0-7 days after symptom onset) and the convalescent phase (>7 days after symptom onset). In the acute phase, the CDC recommends diagnosis using one of two testing combinations: "a nucleic acid amplification test (NAAT) (e.g., RT-PCR) and an IgM antibody test OR an NS1 antigen test and an IgM detection test," but a serum sample is preferred in this stage. However, "a negative result from a RT-PCR or NS1 test does not rule out infection." Furthermore, the CDC recommends that "when the acute (0-7 days) sample is negative in the recommended test combinations or is not available, a convalescent serum sample can be collected and tested." For the convalescent sample, "IgM ELISA is recommended as the primary test after day 8 of symptom onset;" the CDC warns that after day 7 of illness, NAAT or NS1 antigen tests may not be as sensitive for disease detection.

The CDC does not recommend serologic testing by IgG for "diagnosis of acute dengue in patients, as these tests may detect antibodies from dengue infections or other flavivirus infections that occurred in the past."

With regards to specific circumstances, "for people living in or traveling to an area with concurrently circulating flaviviruses, clinicians will need to order plaque reduction neutralization test (PRNT) to rule out dengue on IgM-positive specimens," but PRNT does not always give a conclusive diagnostic result, "particularly in patients that have previously been exposed to more than one flavivirus." Additionally, "if the patient is pregnant and symptomatic and lives in or has traveled to an area with risk of Zika, test for Zika using molecular tests in addition to dengue" (CDC, 2024m).

Zika Virus (CDC, 2024l): The CDC released updated guidelines associated with Zika testing for pregnant individuals. The recommendations for asymptomatic pregnant patients are shown below:

Lived in or traveled to the United States and its territories during pregnancy	Since no confirmed cases of Zika virus have been detected in the United States and its territories since 2018, routine Zika testing is **not** recommended.
Traveled to an area with an active CDC Zika Travel Health Notice during pregnancy	NAAT testing may be considered up to 12 weeks after travel
Traveled to an area with current or past Zika virus transmission outside the U.S. and its territories during pregnancy	Routine testing is not recommended. If the decision is made to test, NAAT testing can be done up to 12 weeks after travel.

Recommendations for symptomatic pregnant patients are shown below:

Lived in or traveled to an area with an active CDC Zika Travel Health Notice during pregnancy OR had sex during pregnancy with someone living in or with recent travel to an area with an active CDC Zika Travel Health Notice	Specimens should be collected as soon as possible after onset of symptoms up to 12 weeks after symptom onset. Perform dengue and Zika virus NAAT and IgM testing on a serum specimen and Zika virus NAAT on a urine specimen. If Zika NAAT is positive and the Zika IgM is negative, repeat NAAT test on newly extracted RNA from same specimen to rule out false-positive results. If both dengue and Zika virus NAATs are negative but either IgM antibody test is positive, confirmatory PRNTs should be performed against dengue, Zika, and other flaviviruses endemic to the region where exposure occurred.
Lived in or traveled to an area with current or past Zika virus	Specimens should be collected as soon as possible after onset of symptoms up to 12 weeks after symptom onset.

transmission during pregnancy	Perform dengue and Zika virus NAAT testing on a serum specimen and Zika virus NAAT on a urine specimen. If Zika NAAT is positive, repeat test on newly extracted RNA from same specimen to rule out false-positive results. Perform IgM testing for dengue only. If dengue NAAT or IgM test is positive, this provides adequate evidence of dengue infection, and no further testing is indicated.
Had sex during pregnancy with someone living in or with recent travel to an area with current or past Zika virus transmission	Specimens should be collected as soon as possible after onset of symptoms up to 12 weeks after symptom onset. Only Zika NAAT should be performed. If Zika NAAT is positive, repeat test on newly extracted RNA from same specimen to rule out false-positive results.

For pregnant patients having a fetus with prenatal ultrasound findings consistent with congenital Zika virus infection, the recommendations are below:

Lived in or traveled during pregnancy to areas with an active CDC Zika Travel Health Notice or current or past Zika virus transmission OR had sex during pregnancy with someone living in or with recent travel to areas with an active CDC Zika Travel Health Notice or current or past Zika virus transmission	Zika virus NAAT and IgM testing should be performed on pregnant person's serum and NAAT on pregnant person's urine. If the Zika virus NAATs are negative and the IgM is positive, confirmatory PRNTs should be performed against Zika and dengue. If amniocentesis is being performed as part of clinical care, Zika virus NAAT testing of amniocentesis specimens should also be performed and results interpreted within the context of the limitations of amniotic fluid testing. Testing of placental and fetal tissues may also be considered.

For symptomatic non-pregnant patients, the recommendations are listed below:

Living in or with recent travel to the United States and its territories	Since no confirmed cases of Zika virus disease have been detected in the United States and its territories since 2018, routine Zika virus testing is not recommended.

Living in or with recent travel to an area with an active CDC Zika Travel Health Notice OR to an area with current or past Zika virus transmission outside the U.S. and its territories	Dengue and Zika virus NAATs should be performed on serum collected ≤7 days after symptom onset. A positive NAAT result typically provides evidence of acute infection. Perform dengue and Zika virus IgM antibody testing on NAAT-negative serum specimens and serum collected >7 days after onset of symptoms. If either dengue or Zika virus IgM antibody testing is positive, and definitive diagnosis is needed for clinical or epidemiologic purposes, confirmatory PRNTs should be performed against dengue, Zika, and other flaviviruses endemic to the region where exposure occurred.

For infants with possible congenital Zika virus infection via gestational parents with possible Zika virus exposure during pregnancy, the CDC recommends to:

- "Collect specimens as soon as possible after birth.
- Zika virus NAAT and IgM testing should be performed on infant serum and NAAT on infant urine.
- If cerebrospinal fluid (CSF) is obtained for other purposes, NAAT and IgM antibody testing should be performed on CSF.
- If the infant's serum is IgM non-negative and NAAT negative, but PRNT was not performed on the gestational parent's serum, PRNT for Zika and dengue viruses should be performed on the infant serum.
- Perform PRNT on a sample collected from an infant aged 18 months or older whose initial sample collected at birth was IgM non-negative and neutralizing antibodies were detected by PRNT in either the infant's or gestational parent's sample."

For asymptomatic non-pregnant patients, "testing for dengue or Zika viruses is **not** recommended for this group" (CDC, 2024l).

Infectious Diseases Society of America (IDSA) and the American Society for Microbiology (American Society of Microbiology)

Laboratory Diagnosis of Tickborne Infections: The information given below outlines the diagnostic procedures for tickborne infections and is taken from Table 50 of the 2024 IDSA/ASM guidelines.

Etiologic Agents	Diagnostic Procedures	Optimum Specimens
Bacteria		
Relapsing fever borreliae ***Borrelia hermsii* (western USA)** ***Borrelia parkeri* (western USA)** ***Borrelia turicatae* (southwestern USA)**	Primary test: Wright's, Giemsa, or Diff-Quik stains of peripheral thin or/ and thick blood smears. Can be seen in direct wet preparation of blood in some cases.	Blood or bone marrow
	Other testing: NAAT, Serologic testing	Serum, blood or body fluids for NAAT. Serum for culture or serologic testing.

Etiologic Agents	Diagnostic Procedures	Optimum Specimens
***Borrelia mazzottii* (southern USA)**		
Borrelia burgdorferi sensu lato complex (Lyme borreliosis) ***Borrelia burgdorferi* (USA)** ***Borrelia mayonii* (USA)** ***Borrelia garinii* (Europe, Asia)** ***Borrelia afzelii* (Europe, Asia)**	Early, localized Lyme disease with erythema migrans (EM) Testing not routinely recommended	Not applicable
	Early if disseminated: If EM or multiple EM rash absent (weeks through months after tick bite) or late (months through years after tick bite) in untreated patients: Primary test: Two-tier testing (acute- and convalescent-phase sera optimal) = EIA antibody screening. If EIA result is positive or equivocal, supplemental IgM/IgG immunoblots or EIAs are required NOTE: Immunoblot or supplemental EIAs should NOT be performed unless an initial EIA is reported as positive or equivocal.	Serum
	Early Lyme Neuroborreliosis: Two-tiered testing algorithm Late Lyme Neuroborreliosis CSF/Serum Antibody Index	Serum Paired serum and CSF, collected within 24 hours
	NAAT	Biopsy specimens of infected skin, synovial fluid or tissue, etc.
***Borrelia miyamotoi (B. miyamotoi* infection, hard tick-borne relapsing fever)**	Primary test for acute infection: NAAT	Blood
	Serology: EIA for detection of antibodies to recombinant GlpQ antigen	Serum
Anaplasma phagocytophilum **(human granulocytotropic anaplasmosis)**	Primary test for acute infection: NAAT Alternate Primary (if experienced technologists available/NAAT is unavailable): Wright or Giemsa stain of peripheral blood or buffy coat leukocytes during week first week of infection.	Blood
	Serology: Acute and convalescent IFA titers for IgG-class antibodies to *A. phagocytophilum* antibodies	Serum
	Immunohistochemical staining of *Anaplasma* antigens in formalin-fixed, paraffin-embedded specimens	Bone marrow biopsies or autopsy tissues (spleen,

Etiologic Agents	Diagnostic Procedures	Optimum Specimens
		lymph nodes, liver, and lung)
Ehrlichia chaffeensis* (human monocytotropic ehrlichiosis)** ***Ehrlichia muris ***Ehrlichia ewingii***	Primary test for acute infection: NAAT NOTE: Only definitive diagnostic assay for *E. ewingii* Wright or Giemsa stain of peripheral blood or buffy coat leukocytes smear during first week of infection	Whole blood for NAAT Blood for Wright or Giemsa stain
	Serology: acute and convalescent IFA titers for *Ehrlichia* IgG-class antibodies NOTE: Not recommended for acute infection	Serum
	Immunohistochemical staining of *Ehrlichia* antigens in formalin-fixed, paraffin-embedded specimens	Bone marrow biopsies or autopsy tissues (spleen, lymph nodes, liver and lung)
***Rickettsia rickettsii* (RMSF)** ***Other spotted fever group Rickettsia* spp (mild spotted fever)** ***R. typhi* (murine typhus)** ***R. akari* (rickettsialpox)** ***R. prowazekii* (epidemic typhus)**	Serology: acute and convalescent IFA for *Rickettsia sp.* IgM and IgG antibodies	Serum
	NAAT	Skin biopsy (preferably a maculopapule containing petechiae or the margin of an eschar) or autopsy tissues (liver, spleen, lung, heart, and brain)
	Immunohistochemical staining of spotted fever group rickettsiae antigens (up to first 24 h after antibiotic therapy initiated) in formalin-fixed, paraffin-embedded specimens	Skin biopsy (preferably a maculopapule containing petechiae or the margin of an eschar) or autopsy tissues (liver, spleen, lung, heart, and brain)
Protozoa		
Babesia microti ***Babesia* sp.**	Primary test: Giemsa, Wright's, Wright-Giemsa stains of peripheral thin and thick blood smears (Giemsa preferred)	Whole blood (EDTA vacutainer tube is a second choice)
	Primary test for acute infection: NAAT	Blood
	Serology: acute and convalescent IFA titers for *Babesia* IgG-class antibodies NOTE: Not recommended for acute infection.	Serum

Etiologic Agents	Diagnostic Procedures	Optimum Specimens
Virus		
Dengue Virus	Serology NS1 Antigen	Serum
	NAAT	CSF, plasma, serum
West Nile Virus and Other Endemic Arboviruses in North America	Serology	Serum
	NAAT	CSF, plasma, serum
Zika Virus	Serology	CSF, serum
	NAAT	CSF, plasma, serum, urine, whole blood

The IDSA/ASM does note that most PCR-based assays for babesiosis only detect *B. microti* even though there are at least three other species of *Babesia* that can cause the infection. "Real time PCR available from CDC and reference labs… Serology does not distinguish between acute and past infection" (Miller et al., 2024).

Their recommendation for the main diagnostic testing for malaria due to *Plasmodium falciparum, P. ovale, P. vivax, P. malariae, and P. knowlesi* is "STAT microscopic examination of Giemsa-stained thick and thin blood films (repeat testing every 12–24 h for a total of 3 exams before ruling out malaria); rapid antigen detection tests followed by confirmatory blood films within 12–24 h." They make the following special remark: "Antigen tests lack sensitivity with low parasitemia and non-*falciparum* malaria and do not differentiate all species. PCR from some reference laboratories will detect and differentiate all species. Calculation of percent parasitemia and species identification (using thick or thin blood films) is required for determining patient management and following response to therapy" (Miller et al., 2024). Concerning DENV, "Plaque reduction neutralization tests (PRNTs) are considered the reference standard for detection of antibodies to arthropod-borne viruses (arboviruses) and provide improved specificity over commercial serologic assays; however, due to the complexity of testing, PRNT is currently only available at select public health laboratories and the CDC." They note that false positives for antibodies to DENV may not necessarily indicate DENV infection since it can also be indicative of a prior flavivirus infection, such as West Nile virus, SLE, or Zika virus. They also state that the "Detection of DENV RNA by NAAT is preferred for acutely ill patients presenting within 7 days of symptom onset. Recently, detection of the DENV NS1 antigen, which is secreted from infected host cells as early as 1 day after symptom onset and up to 10 days thereafter, has become an acceptable alternative to NAAT for diagnosis of acute DENV infection" (Miller et al., 2024).

For West Nile Virus (WNV), they state: "Laboratory diagnosis of these arboviruses is typically accomplished by detecting virus-specific IgM- and/or IgG-class antibodies in serum and/or CSF." Additionally, "However, introduction of blood into the CSF during a traumatic lumbar puncture or defective permeability of the blood-brain barrier may lead to falsely elevated IgM levels in the CSF. Importantly, antibody cross-reactivity among the flaviviruses is not uncommon when using ELISA or IFA-based assays" (Miller et al., 2024).

World Health Organization (WHO)

Interim guidance for laboratory testing of Zika and dengue virus published in July 2022 by WHO includes these updated key considerations, recommendations, and good practices:

- ZIKV and DENV infections need to be differentiated from each other, and from other circulating arboviral and non-arboviral pathogens, using laboratory tests.
- Laboratory tests performed and interpretation of results must be guided by the interval between symptom onset or exposure, and the collection of specimens.
- WHO recommends the use of whole blood, serum, or plasma routine diagnostic testing for arboviruses, and urine for ZIKV NAAT testing.
- Molecular assays are the preferred detection method but the period of RNA detectability following infection is limited.
- Interpretation of serologic test results remains challenging because of cross-reactivity and prolonged detection of virus-specific antibodies; their utility depends on the patient's current and prior flavivirus exposures.
- Testing for antibodies to ZIKV and DENV should thus be done with careful consideration of epidemiologic and clinical context.
- For pregnant women, the diagnosis of ZIKV should always be based on laboratory evidence and testing in these patients should not be limited to a subset of samples, even during outbreaks.
- For pregnant women, accurate diagnosis is of particular importance; prolonged detection of RNA in blood and urine may facilitate. confirmation of ZIKV infection in these patients
- ZIKV IgM testing in pregnant women should be used with caution, since a positive test might reflect infection that occurred prior to pregnancy
- ZIKV testing for asymptomatic pregnant women remains challenging because of unknown optimal timing of specimen collection and risks of false positive and false negative results.
- Only laboratory tests that have undergone independent, comprehensive assessment of quality, safety and performance should be used for diagnosing arboviral infections.
- Any testing for the presence of ZIKV, DENV, and other pathogens in the differential diagnosis should be performed in appropriately equipped laboratories by staff trained in the relevant technical and safety procedures (WHO, 2022a)

American Society for Microbiology (American Society of Microbiology)

The ASM updated guidelines in 2022 on laboratory testing for Zika virus. They state, "Diagnostic testing may be warranted for patients who live in or have recently travelled to an endemic region and are critically ill, hospitalized or pregnant, or infants born to Zika virus positive mothers" (American Society of Microbiology, 2022). The ASM endorses CDC guidelines on Zika as well.

American Academy of Pediatrics 2021-2024 Redbook

Babesiosis (American Academy of Pediatrics, 2021a): "Acute, symptomatic cases of babesiosis typically are diagnosed by microscopic identification of *Babesia* parasites on Giemsa- or Wright-stained blood smears… If the diagnosis of babesiosis is being considered, manual (nonautomated) review of blood smears for parasites should be requested explicitly. If seen, the tetrad (Maltese-cross) form is pathognomonic. *B microti* and other *Babesia* species can be difficult to distinguish…examination of blood smears by a reference laboratory should be considered for confirmation of the diagnosis." They do state that antibody testing can be useful in distinguishing between *Babesia* and *Plasmodium* infections whenever blood smear examinations and travel histories are inconclusive or for detecting individuals with very low levels of parasitemia.

Non-Lyme Borrelia Infections (American Academy of Pediatrics, 2021b): Dark-field microscopy and Wright-, Giemsa-, or acridine orange-stained preparations of blood smears can be used to observe the presence of spirochetes in the initial febrile episode, but their presence is more difficult to determine in

future recurrences. Both enzyme immunoassay and Western immunoblot analysis can detect serum antibodies; however, "Antibody tests are not standardized and are affected by antigenic variations among and within *Borrelia* species and strains." As of publication, PCR and antibody-based testing were still under development and were not widely available.

Ehrlichia, Anaplasma, and Related Infections (American Academy of Pediatrics, 2021e): PCR testing should be performed within the first week of illness to diagnose anaplasmosis, ehrlichiosis, and other *Anaplasmataceae* infections because doxycycline treatment rapidly decreases the sensitivity of PCR. Consequently, negative PCR results do not necessarily indicate a lack of infection. Occasionally, Giemsa- or Wright staining of blood smears can be performed to identify the presence of the morulae of *Anaplasma* in the first week of illness. Culture testing for isolation is not performed. "Serologic testing may be used to demonstrate a fourfold change in immunoglobulin (Ig) G-specific antibody titer by indirect immunofluorescence antibody (IFA) assay between paired acute and convalescent specimens taken 2 to 4 weeks apart. A single mildly elevated IgG titer may not be diagnostic, particularly in regions with high prevalence. IgM serologic assays are prone to false-positive reactions, and IgM can remain elevated for lengthy periods of time, reducing its diagnostic utility."

Rocky Mountain Spotted Fever (RMSF) (American Academy of Pediatrics, 2021h): "The gold standard confirmatory test is indirect immunofluorescence antibody (IFA) to *R rickettsii* antigen. Both immunoglobulin (Ig) G and IgM antibodies begin to increase around 7 to 10 days after onset of symptoms; IgM is less specific, and IgG is the preferred test. Confirmation requires a fourfold or greater increase in antigen-specific IgG between acute (first 1–2 weeks of illness while symptomatic) and convalescent (2–4 weeks later) sera."

Rickettsialpox (American Academy of Pediatrics, 2021g): Rickettsialpox can be mistaken for other rickettsial infections. Ideally, the use of *R. akari*-specific antigen is recommended for serologic diagnosis, but it has limited availability. Otherwise, indirect IFA for *R. rickettsia*, the causative agent of RMSF, since *R. akari* has extensive cross-reactivity. Again, a demonstration of at least a fourfold increase in antibody titers taken two to six weeks apart is indicative of infection.

Chikungunya (American Academy of Pediatrics, 2021c): "Laboratory diagnosis generally is accompanied by testing serum to detect virus, viral nucleic acid, or virus-specific immunoglobulin (Ig) M and neutralizing antibodies." RT-PCR can be used to diagnose chikungunya during the first week after onset of symptoms since chikungunya-specific antibodies have not formed at that time. After the first week, serum testing of IgM or a plaque reduction neutralization test can be performed.

Dengue (American Academy of Pediatrics, 2021d): "Dengue virus is detectable by RT-PCR or NS1 antigen EIAs from the beginning of the febrile phase until day 7 to 10 after illness onset." Cross-reactivity occurs between anti-dengue virus IgM and other flaviviruses, including Zika. IgG EIA and hemagglutination testing is not specific for diagnosis of dengue, and IgG antibodies remain elevated for life; consequently, a fourfold increase in IgG between the acute and convalescent phase can confirm recent infection, with "Reference testing is available from the Dengue Branch of the Centers for Disease Control and Prevention."

Malaria (American Academy of Pediatrics, 2021f): Microscopic identification of *Plasmodium* on both thick and thin blood films should be performed. "If initial blood smears test negative for *Plasmodium* species but malaria remains a possibility, the smear should be repeated every 12 to 24 hours during a 72-hour period… Serologic testing generally is not helpful, except in epidemiologic surveys… Species confirmation and antimalarial drug resistance testing are available free of charge at the Centers for

Disease Control and Prevention (CDC) for all cases of malaria diagnosed in the United States." One FDA approved RADT is available in the U.S. to hospitals and commercial labs; however, both positive and negative test results must be corroborated by microscopic examination.

West Nile Virus (WNV) (American Academy of Pediatrics, 2021i): PCR is not recommended for diagnosis of WNV in immunocompetent patients since WNV RNA is usually no longer detectable by the initial onset of symptoms. "Detection of anti-WNV immunoglobulin (Ig) M antibodies in serum or CSF is the most common way to diagnose WNV infection." Anti-WNV IgM levels can remain elevated for longer than one year so a positive test result may be indicative of a prior infection. "Plaque-reduction neutralization tests can be performed to measure virus-specific neutralizing antibodies and to discriminate between cross-reacting antibodies from closely related flaviviruses. A fourfold or greater increase in virus-specific neutralizing antibodies between acute-and convalescent-phase serum specimens collected 2 or 3 weeks apart may be used to confirm recent WNV infection."

International Encephalitis Consortium (IEC)

In 2013, the IEC released their *Case Definitions, Diagnostic Algorithms, and Priorities in Encephalitis*. Concerning arboviruses, they state the following: "For most arboviruses, serologic testing of serum and CSF is preferred to molecular testing, since the peak of viremia typically occurs prior to symptom onset. For example, in patients with West Nile virus (WNV) associated with neuroinvasive disease, CSF PCR is relatively insensitive (57%) compared with detection of WNV IgM in CSF. The cumulative percentage of seropositive patients increases by approximately 10% per day during the first week of illness suggesting the need for repeat testing if the suspicion for disease is strong in those with initially negative results. Notably, arbovirus IgM antibodies may be persistently detectable in the serum and, less commonly, in the CSF, for many months after acute infection, and therefore may not be indicative of a current infection. Therefore, if possible, documentation of acute infection by seroconversion and/or 4-fold or greater rises in titre using paired sera is recommended" (Venkatesan et al., 2013).

Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

On 6/29/2017, the FDA approved the Rickettsia Real-Time PCR Assay (K170940) by the Centers for Disease Control and Prevention (CDC) with the following definition: "An in vitro diagnostic test for the detection of Rickettsia spp. nucleic acids in specimens from individuals with signs or symptoms of rickettsial infection and epidemiological risk factors consistent with potential exposure. Test results are used in conjunction with other diagnostic assays and clinical observations to aid in the diagnosis infection, in accordance with criteria defined by the appropriate public health authorities in the Federal government" (FDA, 2018).

On 9/1/2009, the FDA approved the BinaxNOW Malaria Positive Control Kit (K083744) rapid diagnostic test (RDT), an in vitro qualitative immunochromatographic assay, for use by hospital and commercial laboratories, but it is not approved for individual or physician offices (FDA, 2018; Tan & Abanyie, 2024).

As of 8/7/2018, the FDA has approved the following assays for the detection of West Nile Virus (FDA, 2018): West Nile Virus ELISA IgG model EL0300G and West Nile Virus IgM Capture ELISA model EL0300M by Focus Technologies, Inc., West Nile Virus IgM Capture ELISA model E-WNV02M and West Nile Virus IgG Indirect ELISA by Panbio Limited, West Nile Detect IgM ELISA by Inbios Intl, Inc., Spectral West Nile Virus IgM Status Test by Spectral Diagnostics, Inc., and the EUROIMMUN Anti-West Nile Virus ELISA (Biggs et al.) and EUROIMMUN Anti-West Nile Virus ELISA (IgM) by Euroimmun US, Inc.

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
86280	Hemagglutination inhibition test (HAI)
86382	Neutralization test, viral
86619	Antibody; Borrelia (relapsing fever)
86666	Antibody; Ehrlichia
86750	Antibody; Plasmodium (malaria)
86753	Antibody; protozoa, not elsewhere specified
86757	Antibody; Rickettsia
86788	Antibody; West Nile virus, IgM
86789	Antibody; West Nile virus
86790	Antibody; virus, not elsewhere specified
86794	Antibody; Zika virus, IgM
87040	Culture, bacterial; blood, aerobic, with isolation and presumptive identification of isolates (includes anaerobic culture, if appropriate)
87207	Smear, primary source with interpretation; special stain for inclusion bodies or parasites (eg, malaria, coccidia, microsporidia, trypanosomes, herpes viruses)
87449	Infectious agent antigen detection by immunoassay technique, (eg, enzyme immunoassay [EIA], enzyme-linked immunosorbent assay [ELISA], fluorescence immunoassay [FIA], immunochemiluminometric assay [IMCA]) qualitative or semiquantitative; not otherwise specified, each organism
87468	Infectious agent detection by nucleic acid (DNA or RNA); Anaplasma phagocytophilum, amplified probe technique
87469	Infectious agent detection by nucleic acid (DNA or RNA); Babesia microti, amplified probe technique
87478	Infectious agent detection by nucleic acid (DNA or RNA); Borrelia miyamotoi, amplified probe technique
87484	Infectious agent detection by nucleic acid (DNA or RNA); Ehrlichia chaffeensis, amplified probe technique

CPT	Code Description
87662	Infectious agent detection by nucleic acid (DNA or RNA); Zika virus, amplified probe technique
87798	Infectious agent detection by nucleic acid (DNA or RNA), not otherwise specified; amplified probe technique, each organism
87899	Infectious agent antigen detection by immunoassay with direct optical (ie, visual) observation; not otherwise specified
0043U	Tick-borne relapsing fever Borrelia group, antibody detection to 4 recombinant protein groups, by immunoblot, IgM Proprietary test: Tick-Borne Relapsing Fever Borrelia (TBRF) ImmunoBlots IgM Test Lab/Manufacturer: IGeneX Inc
0044U	Tick-borne relapsing fever Borrelia group, antibody detection to 4 recombinant protein groups, by immunoblot, IgG Proprietary test: Tick-Borne Relapsing Fever Borrelia (TBRF) ImmunoBlots IgG Test Lab/Manufacturer: IGeneX Inc

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

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Revision History

Revision Date	Summary of Changes
09/04/2024	Reviewed and Updated: Updated background, guidelines, and evidence-based scientific references. Literature review necessitated the following changes in coverage criteria: NAAT defined in CC1 Edited for clarity and consistency: Former CC3, now CC5. Former CC5a, now CC7a. Former CC17, now CC19. Prior to recent CDC updates, most Borrelia spp. were considered to cause tickborne relapsing fever. The CDC has now separated this into hard tick relapsing fever and soft tick relapsing fever (may still be called tickborne relapsing fever). HTRF and STRF, as well as LBRF (also caused by Borrelia spp), have different testing recommendations. For clarity and consistency, former CC13 and CC14 become CC3 and CC4 and have been reorganized to discuss the recommended testing for relapsing fevers caused by Borrelia spp. CC3 and CC4 now read: "3) For individuals suspected of having a relapsing fever caused by a Borrelia spp., the following testing MEETS COVERAGE CRITERIA: a) For individuals suspected of having hard tick relapsing fever (HTRF) (see Note 2): serologic assays to detect Borrelia antibodies or PCR testing to detect Borrelia miyamotoi. b) For individuals suspected of having louse-borne relapsing fever (LBRF) (see Note 3): peripheral blood smear microscopy or PCR testing to detect Borrelia recurrentis. c) For individuals suspected of having a soft tick relapsing fever (STRF)/tickborne relapsing fever (TBRF) (see Note 4): dark-field microscopy of a peripheral blood smear, microscopy of a Wright- or Giemsa-stained blood smear, PCR testing to detect Borrelia spp., or serologic assays to detect Borrelia antibodies. 4) For individuals suspected of having a relapsing fever caused by a Borrelia spp., culture testing for Borrelia DOES NOT MEET COVERAGE CRITERIA." Results in new Note 2 and Note 3 to define signs/symptoms of HTRF and LBRF, Note 9 becomes Note 4, defines STRF/TBRF. All other notes shift in numbering. Testing indications for CTF updated to include PCR testing. CC7b, formerly CC5b, updated from "non-pregnant individuals" to "individuals", as CDC guideline updates indicate that all individuals with signs/symptoms of Zika should be tested for DENV. Now reads: "b) For individuals who are symptomatic for Zika virus infection (see Note 8)." New CC12: "12) To confirm the species of Plasmodium in an individual diagnosed with malaria, PCR testing MEETS COVERAGE CRITERIA." Results in a change to former CC10, now CC13, which did not allow NAAT for Plasmodium. Now reads: "13) For individuals suspected of having malaria (see Note 10), the use of IFA for Plasmodium antibodies DOES NOT MEET COVERAGE CRITERIA." Former CC11, now CC14, changed "limit to two units" to "two tests occurring a minimum of two weeks apart". Now reads: "14) For individuals suspected of having a rickettsial disease (see Note 11), the use of an IFA assay for IgG antibodies (two tests occurring a minimum of two weeks apart) MEETS COVERAGE CRITERIA. Former CC15, now CC16, added IFA for IgG as an allowed test. Now reads: "16) For individuals suspected of having West Nile virus (WNV) disease (see Note 12), the use of IFA for WNV-specific IgG or IgM antibodies in either serum or CSF and a

	confirmatory plaque reduction neutralization test for WNV MEETS COVERAGE CRITERIA." New CC17: "17) To confirm a WNV infection in individuals who are immunocompromised, nucleic acid detection of WNV MEETS COVERAGE CRITERIA." These two updates results in a change to former CC16, now CC18. Now reads: "18) For immunocompetent individuals suspected of having WNV disease (see Note 12), the use of NAAT for WNV DOES NOT MEET COVERAGE CRITERIA." Fromer CC18,19, and 20, now CC20, 21, and 22, edited based on CDC guideline updates for Zika virus testing recommendations. Now read: "20) For the detection of Zika virus, the use of NAAT MEETS COVERAGE CRITERIA in the following individuals: a) Up to 12 weeks after the onset of symptoms for symptomatic (see Note 8) pregnant individuals who, during pregnancy, have either lived in or traveled to areas with current or past Zika transmission or who have had sex with someone who either lives in or has recently traveled to areas with current or past Zika virus transmission (see Note 14). b) For symptomatic non-pregnant individuals living in or with recent travel to an area with an active CDC Zika Travel Health Notice or an area with current or past Zika virus transmission (see Note 14) when symptoms presented within the last seven days. 21) Zika virus NAAT and Zika virus IgM testing, as well as a confirmatory plaque reduction neutralization test for Zika, MEETS COVERAGE CRITERIA in any of the following situations: a) Up to 12 weeks after the onset of symptoms for symptomatic (see Note 8) pregnant individuals who, during pregnancy, have either lived in or traveled to areas with an active CDC Zika Travel Health Notice or who have had sex with someone who either lives in or has recently traveled to areas with an active CDC Zika Travel Health Notice (see Note 14). b) For pregnant individuals who have a fetus with prenatal ultrasound findings consistent with congenital Zika virus infection (see Note 15). c) For infants born from individuals who, during pregnancy, tested positive for Zika virus. d) For infants born with signs and symptoms of congenital Zika syndrome (see Note 15) and who have a birthing parent who had a possible Zika virus exposure during pregnancy. e) For symptomatic non-pregnant individuals living in or with recent travel to an area with an active CDC Zika Travel Health Notice or an area with current or past Zika virus transmission (see Note 14) when symptoms presented more than seven days prior to testing. 22) For non-pregnant individuals who have not traveled outside of the United States and its territories and who are symptomatic for Zika virus infection (see Note 8), NAAT and/or IgM testing for Zika detection DOES NOT MEET COVERAGE CRITERIA. Former Note 4, now Note 7, edited to update signs and symptoms. Former Note 9, now Note 4, updated name from TBRF to STRF/TBRF, updated causative pathogens, updated signs and symptoms. Former Note 12, now Note 14, updated with CDC classifications of Zika risk.

Testing of Homocysteine Metabolism-Related Conditions

Policy Number: AHS – M2141 – Testing of Homocysteine Metabolism-Related Conditions	Initial Presentation Date: 12/12/2016 Effective Date: 4/1/2025

Policy Description

Homocystinuria is a metabolic condition in which the body is unable to properly process certain amino acids, resulting in an abnormal accumulation of homocysteine and its metabolites in the blood and urine (NIH, 2023). Homocystinuria is primarily due to genetic causes; however, homocystinuria can also be due to non-genetic causes, including severe deficiency of vitamin B12, also known as cobalamin (Mudd et al., 2000).

Related Policies

Policy Number	Policy Title
AHS-G2014	Vitamin B12 And Methylmalonic Acid Testing
AHS-G2035	Prenatal Screening (Nongenetic)
AHS-G2050	Cardiovascular Disease Risk Assessment
AHS-M2041	Venous and Arterial Thrombosis Risk Testing
AHS-M2180	Genetic Markers for Assessing Risk of Cardiovascular Disease

Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" of this policy document.

1) Newborn screening for homocysteine-related conditions **MEETS COVERAGE CRITERIA** in **any** of the following situations:

   a) Screening for classic homocystinuria due to CBS deficiency by performing quantitative plasma amino acids analysis and/or plasma or urine total homocysteine analysis.

   b) Screening for homocystinuria in dried blood spots.

   c) Screening for hypermethioninemia in dried blood spots.

2) When the initial screening test result exceeds the cut-off level of methionine, a repeat dried blood specimen submitted to the newborn screening program, **or** a quantitative plasma amino acid analysis and analysis of plasma total homocysteine **MEETS COVERAGE CRITERIA**.

3) For the diagnosis of phenotype variants of classic homocystinuria due to CBS deficiency, the pyridoxine (B6) challenge test **MEETS COVERAGE CRITERIA**.

4) For individuals over 18 years of age with homocystinuria suspected to be caused by CBS deficiency **and** for monitoring therapy in those with confirmed CBS deficiency, total homocysteine testing in plasma **MEETS COVERAGE CRITERIA**.

5) Plasma free homocysteine testing **DOES NOT MEET COVERAGE CRITERIA.**

Table of Terminology

Term	Definition
3-MST	3-mercaptopyruvate
ACMG	American College of Medical Genetics
cblC	Homocystinuria type C
cblD	Cobalamin D
cblD-Hcy	Cobalamin D homocysteine
CblE	Methylcobalamin type E
cblF	Cobalamin F
cblG	Methylcobalamin type G
cblJ	Methylcobalamin type J
CBS	*Cystathionine β-synthase*
CLIA '88	Clinical Laboratory Improvement Amendments of 1988
CMS	Centers for Medicare and Medicaid
CT	Computed tomography
HT	Heterozygous state
CTH	Cystathionine
DNA	Deoxyribonucleic acid
EDTA	Chelating agentin deoxyribose nucleic acid extraction
E-HOD	European Network and Registry for Homocystinuria and Methylation Defects
FDA	Food and Drug Administration
FPIA	Fluorescence polarization immunoassay
GCLC	Glutamylcysteine
GC-MS	Gas chromatography–mass spectrometry

Hcy	Homocysteine
HHS	The U.S. Department of Health and Human Services
HPLC	High performance liquid chromatography
LC-MS	Liquid chromatography–mass spectrometry
LC-MS-MS	Liquid chromatography tandem mass spectrometry
LDTs	Laboratory-developed tests
MENA	Middle East and North Africa
Met	Methionine
MMA	Methylmalonic acid
MMADHC	Methylmalonic aciduria and homocystinuria type D protein
MRI	Magnetic resonance imaging
MTHFR	Methylene tetrahydrofolate reductase
MTR	Methionine synthase
MTRR	Methionine synthase reductase
NIH	National Institutes of Health
Phe	Phenylalanine
SAM	S-adenosyl methionine
tHcy	Total homocysteine
TT	Homozygous state
UPLC-MS/MS	Ultra performance liquid chromatography-tandem mass spectrometry

Scientific Background

Homocysteine (Hcy), a naturally occurring intermediary amino acid, is involved in multiple metabolic pathways, including the transsulfuration pathway as well as methionine (Met) metabolism. Classic homocystinuria, which results in an accumulation of Hcy and its metabolites in the blood and urine, is due to genetic mutations in *cystathionine-β-synthase* (*CBS*). CBS is the enzyme responsible for the rate-limiting step of the transsulfuration pathway and is dependent on pyridoxine (vitamin B6) (Zhu et al., 2018). If this enzyme is blocked, the transsulfuration of Hcy and the accumulation of both Hcy and Met will be limited, as Met concentration is enhanced by remethylation. The disruption of the Met metabolic pathway, as shown in Figure 1 below (Zhu et al., 2018), prevents Hcy from being used properly; this creates in a buildup of Hcy and toxic by-products in the blood, with excess Hcy excreted in urine (Mazaheri et al., 2017).

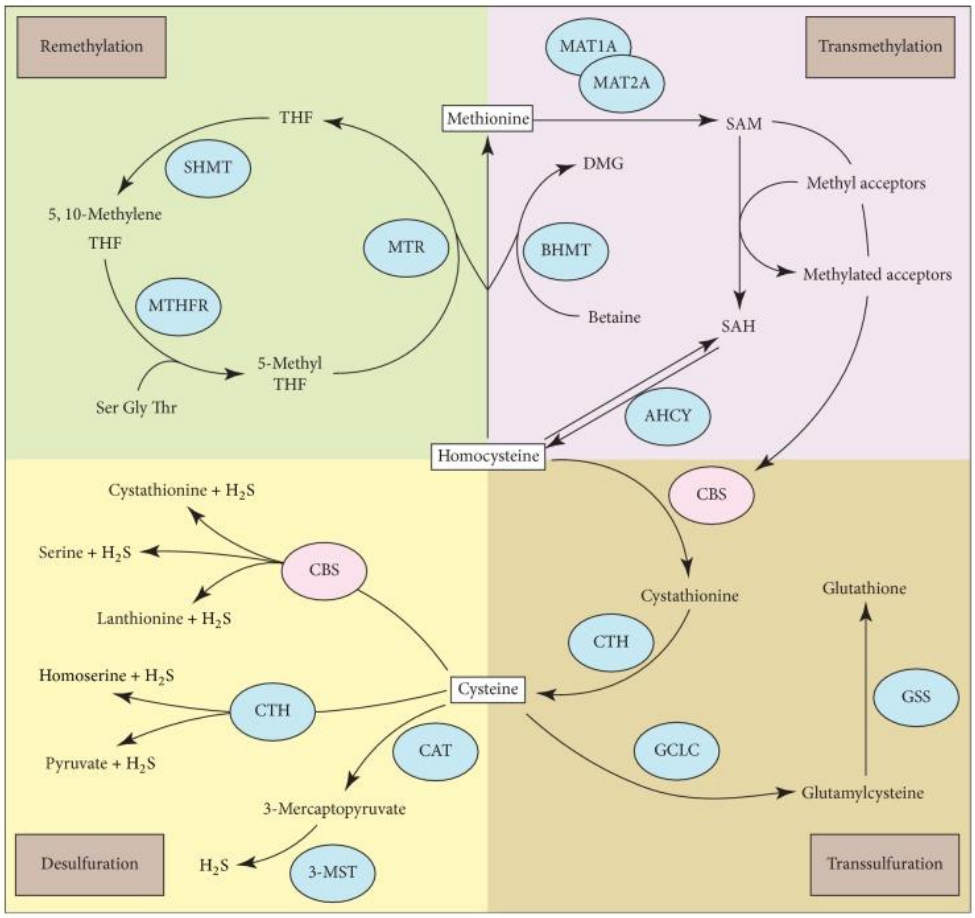

Figure 1: Homocysteine is a common metabolite linked to multiple metabolic pathways, including methionine/S-adenosyl methionine (SAM) metabolism, transsulfuration, and desulfuration. Figure taken from (Zhu et al., 2018).

Homocystinuria due to CBS deficiency can cause eye problems, skeletal abnormalities, an increased risk for blood clots, and developmental delay. Homocystinuria may also generate white matter abnormalities in the brain, potentially mimicking other disorders such as leukoencephalopathy when imaged with computed tomography (CT) and magnetic resonance imaging (MRI) scans (Ismayilova et al., 2019; Li et al., 2018).

The exact incidence of homocystinuria due to CBS deficiency is unknown. In 1985, the incidence was estimated to be around 1:344,000 worldwide (Mudd et al., 1985). However, the National Institutes of Health (NIH) is now estimating these rates to be much higher, around 1:150,000 worldwide, 1:200,000-300,000 in the United States, and 1:1,800 in Qatar (NIH, 2023). In European populations, incidence rates have been predicted by molecular epidemiological studies to be between 1:6,400 and 1:20,500 (Gaustadnes et al., 1999; Janosík et al., 2009). Higher prevalence in the MENA (Middle East and North Africa) region could be attributed to high consanguinity in those communities (Al-Sadeq & Nasrallah, 2020). Infants with homocystinuria due to CBS deficiency are asymptomatic at birth, with symptoms slowly developing if left untreated. However, these symptoms are highly variable. Some affected patients may exhibit mild symptoms of the disorder while others may develop potentially life-threatening complications. Depending on the population affected and type of *CBS* gene mutation, symptoms can be as severe as ectopia lentis, Marfanoid features, mental retardation, idiopathic infertility, osteoporosis,

and severe premature atherosclerosis (Al-Sadeq & Nasrallah, 2020; Rosenson et al., 2024). The phenotype of these patients mainly relates to pyridoxine-responsiveness: pyridoxine treatment responders exhibit a milder phenotype and a later onset than pyridoxine treatment nonresponders (Abbott et al., 1987; Mudd et al., 1985). Early detection and treatment is important in preventing or reducing the severity of the disorder. Screening for homocystinuria is frequently incorporated into state newborn screening programs (Rose & Dolan, 2012). While a newborn blood spot specimen for hypermethioninemia will detect homocystinuria due to CBS deficiency in some, not all affected individuals will be detected by this test (Sacharow et al., 2004).

According to Sacharow et al. (2004), the biochemical features of homocystinuria include:

- Markedly increased concentrations of total homocysteine, plasma homocysteine, homocysteine-cysteine mixed disulfide, and methionine
- Increased concentration of homocysteine in urine
- Reduced CBS enzyme activity

Classical biochemical findings establishing the diagnosis are summarized in the following table titled: Cardinal Biochemical Findings that Establish the Diagnosis of Homocystinuria (Sacharow et al., 2004).

Analyte	**Specimen**	**Expected Findings**		
		Neonate with homocystinuria	**Untreated older individual with homocystinuria**	**Control**
Total homocysteine (tHcy)	Plasma	50 to >100 µmol/L	>100 µmol/L	<15 µmol/L
Methionine (on amino acid analysis)	Plasma	200-1500 µmol/L (3-23 mg/dL)	>50 µmol/L (>0.7 mg/dL)	10-40 µmol/L (0.2-0.6 mg/dL)

Homocystinuria due to genetic causes is inherited in an autosomal recessive pattern. Many different forms of homocystinuria can occur and signs and symptoms vary depending on the gene mutation. *CBS* gene mutations cause the most common form of homocystinuria. This mutation is referred to as "classic" homocystinuria or CBS deficiency. Other gene mutations that can result in homocystinuria include *MTHFR*, *MTR*, *MTRR*, and *MMADHC*. The *MTHFR*, *MTR*, and *MTRR* genes all revolve around the remethylation pathway of Hcy, while the *MMADHC* gene plays a role in Vitamin B12 metabolism (Froese et al., 2015; Wang et al., 2016).

Homocystinuria may also be associated with a diagnosis of methylmalonic acidemia, when the body cannot efficiently break down specific fats or proteins, leading to a methylmalonic acid buildup in the blood. Methylmalonic aciduria and homocystinuria type C (cblC) is characterized by a vitamin B12 disorder initiated by a mutation in the *MMACHC* gene; symptoms of this disorder fall into several categories, including thromboembolic and neurological issues such as cognitive and psychiatric episodes (Collison et al., 2015).

Analytical Validity

This concentration of total homocysteine (tHcy) in blood plasma is the primary clinical analyte measured to diagnose homocystinuria. A study using liquid chromatography–mass spectrometry (LC-MS) calculated limits of detection (0.06 µmol/L) and quantification (0.6 µmol/Lu) of tHcy (Nelson et al., 2003). Another study using gas chromatography–mass spectrometry (GC-MS) found a detection limit of 0.4 µmol/L as well as intra- and inter-run variations of five and eight percent, respectively. Furthermore, this method was found to compare well with the LC-MS-MS method; the GC-MS method had a mean difference of -0.4 µmol compared to the LC-MS-MS method (Belkhiria et al., 2007). Fluorescence polarization immunoassay (FPIA) was found to compare favorably to the high performance liquid chromatography (HPLC) and MS approaches as well (five percent imprecision with negative two percent to three percent bias) so it is a practical option if the more precise approaches are not available; unfortunately, this study only measured levels up to 45 µmol/L, whereas severe homocystinuria can exceed 100 µmol/L (Nexo et al., 2000).

More recently, Concepción-Alvarez et al. (2016) have validated a method to quantify Hcy in plasma samples via HPLC. Hcy levels were measured in a total of 46 patients and the authors found that HPLC was able to "identify and quantify Hcy without interferences" and that the identified detection limit was 3.12 µM and quantification limit 6.25 µM (Concepción-Alvarez et al., 2016). This research has provided further validation for Hcy plasma testing in ailments such as homocystinuria where this amino acid is increased.

For the detection of Hcy-related conditions, methylmalonic acid and tHcy are commonly measured in both plasma samples and dried blood spots. Using ultra performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS), de Sain-van der Velden et al. (2015) recently compared methylmalonic acid and tHcy levels collected either from a dried blood spot or from plasma concentration testing methods to determine which is the more efficient and accurate method. The authors note that the plasma assay performed better than the dried blood spot testing method in most areas, but that dried blood spot testing was superior for tHcy stability. Furthermore, a strong correlation of tHcy was found in both testing methods, ($y=0.46\pm1.12$ ($r(2)=0.91$)), leading to the authors suggestion that tHcy testing in plasma can be replaced by tHcy in dried blood spots (de Sain-van der Velden et al., 2015).

Clinical Utility and Validity

A diagnosis of classic homocystinuria (caused by CBS deficiency) is established by measurement of tHcy. The normal level is <15 µMol/L, whereas a newborn with homocystinuria is expected to measure at >50 µMol/L and an older, untreated individual will likely measure at >100 µMol/L (Sacharow et al., 2004). A measurement of Met in plasma can corroborate a diagnosis, as the metabolic pathway involves a buildup of Met in addition to the buildup of Hcy (Sacharow et al., 2004). While free Hcy composes about 15-25% of tHcy levels, separate free Hcy testing is unnecessary: tHcy measurement already includes all forms of Hcy (Rosenson et al., 2024).

The detection of biallelic pathogenic variants in CBS can substantiate a diagnosis of classic homocystinuria (Sacharow et al., 2004). There are two phenotypic variants in homocystinuria, both caused by *CBS*: B6-responsive and B6-non-responsive homocystinuria. The pyridoxine (B6) challenge test is performed to determine the variant and if vitamin B6 therapy will be beneficial (Sacharow et al., 2004). Testing for homocystinuria usually involves biochemical testing in urine and/or genetic testing for known mutations. Genetic testing can be done using a single gene or multi-gene panel which may

include sequence analysis, deletion/duplication analysis, and/or other non-sequencing-based tests. Homocystinuria typically involves CBS deficiency and while the activity of the CBS enzyme could be performed in cultured fibroblasts when genetic tests are inconclusive, enzymatic testing for CBS deficiency is no longer available in USA (Sacharow et al., 2004).

Methylene tetrahydrofolate reductase (*MTHFR*) mutations are of interest in homocystinuria. The MTHFR enzyme catalyzes the reduction of 5,10-methylenetetrahydrofolate to 5,10-methyltetrahydrofolate, the methyl donor for the conversion of Hcy to Met. Failure of this enzyme (<20% of normal levels) leads to increased Hcy and Met, as well as the production of other symptoms associated with homocystinuria (Long & Goldblatt, 2016). The two most common mutations in the *MTHFR* gene are 677T (changed from a C nucleotide) and 1298C (changed from an A). Both mutations can be heterozygotic or homozygotic, and both can lead to loss of enzymatic function (Gonzales et al., 2017). The 677T mutation is more severe, as in the homozygous state (TT) it results in up to 70% loss of enzymatic function, compared to only a 35% loss of function in the heterozygous state (CT) (Frosst et al., 1995). The 1298C mutation results in a loss of enzymatic function; 30% and 15% for its homozygous and heterozygous forms, respectively (Weisberg et al., 1998).

However, it is possible that dietary factors (notably low levels of folate or Vitamin B12) influence tHcy levels more than genetic factors. A study covering 452 young adults found tHcy variance to have a nine percent total genetic contribution (i.e. genetic polymorphisms) compared to a 35% contribution from dietary factors. The only polymorphism found to have a significant effect on tHcy levels was the 677T mutation, which interacted with low folate levels to produce a high tHcy phenotype. Compared to the authors earlier studies of genetic influence on tHcy levels, the younger cohort's genetic contribution on tHcy levels was measured out to be higher than the older cohort's (nine percent compared to seven percent for the older cohort). Furthermore, the authors suggest that genetic influence on tHcy levels are more pronounced during early life and environmental factors are more influential as time passes (Gaughan et al., 2001; Harmon et al., 1999; Kluijtmans et al., 2003).

Another study conducted by Gales et al. (2018) found that focal epilepsy presentation in the context of adult or adolescent onset could result from a mutation leading to *MTHFR* deficiency. It is critical that this mutation found in homocystinuria be detected early for treatment, as the neuropsychiatric syndrome could be easily treated with a combination of vitamin B9, vitamin B12, and betaine (Gales et al., 2018).

A novel newborn screening method has been developed by researchers: a two-tier algorithm using a methionine (Met) to phenylalanine (Phe) ratio. Data from 125,047 neonates was utilized to determine this accuracy of this method (Okun et al., 2017). It was reported that "Met to Phe ratio was found to be more effective for first sieve than Met, sorting out nearly 90% of normal samples. Only 10% of the samples would have to be processed by second-tier measurement of Hcy in dried blood spots" (Okun et al., 2017). This novel testing method resulted in 100% sensitivity and specificity for classic homocystinuria newborn screening (Okun et al., 2017).

Guo et al. (2022) investigated the effects of maternal Hcy concentrations, *MTHFR*, and *MTRR* gene presence on the occurrence of fetal aneuploidy through a retrospective case-control study. From the differences in the maternal *MTHFR* 677C>T, *MTHFR* 1298A>C and *MTRR* 66A>G genetic polymorphisms and maternal Hcy concentrations between aneuploidy and control mothers, they found that the mutations were associated with multiple trisomies, including Down syndrome (Trisomy 21), trisomy 15, and Turner syndrome. The 677C>T polymorphism was associated with the most trisomies and transfer genes. Lastly, there was an increased concentration of Hcy among mothers of fetuses with trisomies 22, 21, 18, 16, and 15 and Turner syndrome in comparison to the control mothers. Due to the identified

associations, it becomes even more crucial to study effects of combining maternal genetic factors and body systemic factors like homocysteine concentration on spontaneous fetal aneuploidy formation that were not previously understood (Guo et al., 2022).

Guidelines and Recommendations

The United States Department of Health and Human Services

The Secretary of the U.S. Department of HHS has developed a recommended uniform screening panel for every universal newborn screening program; the amino acid disorder homocystinuria is recommended as a core condition for newborn screening, and the organic acid condition methylmalonic acidemia with homocystinuria is recommended as a secondary screening condition. Methylmalonic acidemia due to methylmalonyl-CoA mutase or cobalamin disorders is included as a core condition as well (HHS, 2024).

American College of Medical Genetics (ACMG)

The American College of Medical Genetics recommends quantitative testing of plasma amino acids to determine increased levels of Hcy and Met; classic homocystinuria is characterized by increases in both Hcy and Met, while increased Met may be indicative of other disorders (ACMG, 2021b). Also, plasma Hcy analysis will show increased Hcy in classic homocystinuria and normal or only slightly increased Hcy in the other disorders. Urine Hcy will be significantly increased in classic homocystinuria (ACMG, 2021b).

In the Confirmatory Algorithms for Met, ACMG indicates that increased Met and increased tHcy are indicative of homocystinuria due to CBS deficiency (ACMG, 2021a).

European Network and Registry for Homocystinuria and Methylation Defects (E-HOD)

In 2015, a project by the E-HOD released the *Newborn Screening for Homocystinurias and Methylation Disorders: Systematic Review and Proposed Guidelines*. In this guideline, authors recommend newborn screening for CBS deficiency by detecting elevated Met, methionine-to-phenylalanine ratio, and/or tHcy in dried blood spots. Specificity is increased by analyzing tHcy as a second-tier marker and calculating Met/tHcy ratio is also suggested (Huemer et al., 2015)

Newborn screening for the cblD-Hcy, CblE, and cblG defects, and for *MTHFR* deficiency could be possible by measuring Met and methionine-to-phenylalanine ratio in dried blood spots followed by analysis of tHcy as a second-tier marker. However, it is stated that the efficacy and feasibility of screening for these disorders is largely unknown (Huemer et al., 2015).

As a part of E-HOD, the *Guidelines for Diagnosis and Management of The Cobalamin-Related Remethylation Disorders cblC, cblD, cblE, cblF, cblG, cblJ and MTHFR Deficiency* were released in 2017. Huemer et al. (2017) "strongly recommend measuring plasma total homocysteine in any patient presenting with the combination of neurological and/or visual and/or haematological symptoms, subacute spinal cord degeneration, atypical haemolytic uraemic syndrome or unexplained vascular thrombosis." For a "valid, timely laboratory diagnosis," the authors also add:

- "We strongly recommend that investigations in patients with a suspected remethylation disorder should start with the measurement of total homocysteine in blood. We recommend the blood sample for tHcy to be centrifuged within an hour and kept at four degrees or frozen until analysis.

Immunoassays or chromatographic methods are suitable for tHcy measurement. (Quality of the evidence: moderate)
- We strongly recommend against measuring free homocysteine instead of total homocysteine. (Quality of the evidence: moderate)
- We strongly recommend that in the case of high total homocysteine, plasma and urine samples for determination of MMA, methionine, folate and vitamin B12 are to be obtained before treatment is started. (Quality of the evidence: moderate)" (Huemer et al., 2017).

Another guideline written as a part of E-HOD provides practical guides to recognition, diagnosis and management of CBS deficiency. The guideline presented 41 separate recommendations based on a literature review by the Guideline Development Group and the authors admitted that the quality of the identified data was poor and many of their recommendations were grade D; however, the highest recommendation was given to measuring the plasma total homocysteine concentrations in any patient whose signs and symptoms strongly suggest the diagnosis (Morris et al., 2017).

For the biochemical diagnosis, a tHcy test is recommended as "the frontline test" for the diagnosis of CBS deficiency. Plasma free Hcy is only detectable at tHcy concentrations above 50-60 µmol/L; its measurement is not particularly sensitive or even reproducible and is, therefore, not recommended. Untreated patients with a CBS deficiency typically have tHcy concentrations above 100 µmol/L and a diagnosis is likely if an elevated tHcy is found along with high or borderline high plasma Met concentrations. Further information such as low plasma cystathionine concentration or increased Met:Cystathionine ratio can support a diagnosis. Finally, tHcy measurement using dried blood spots can be done if plasma processing is not possible.

The European Network and Registry for Homocystinuria and Methylation Defects recommends confirming CBS deficiency by measuring cystathionine synthase activity in fibroblasts or plasma and/or by mutation analysis of CBS gene. The gold standard for confirming CBS deficiency is determination of cystathionine production of Hcy and serine in cultured fibroblasts. Either the enzyme or DNA can be analyzed and if one method does not confirm a diagnosis, the other method should be done. The grade of this recommendation is B-C.

Despite technical pitfalls of DNA testing, E-HOD recommends a molecular genetic analysis of the CBS gene for the confirmation of CBS deficiency and for carrier and prenatal testing (grade B). For the prenatal diagnosis, the molecular analysis is a preferred technique during the first trimester of pregnancy. If the mutations are known in the family, enzyme analysis can be performed in cultured amniocytes, but not in chorionic villi. Preimplantation analysis could also be done (grade C-D).

For newborn screening, it is recommended to increase specificity of Met testing by using tHcy as a second marker and calculating Met/tHcy ratio (grade C). Several other metabolic disorders can cause an increased Met concentration and the exact sensitivity of detecting Met in newborns with a CBS deficiency is unknown. Although the median Met concentration of CBS deficient patients is far greater than the median of a healthy neonate (103 µmol/L compared to 20 µmol/L), individual Met values may still vary.

Screening for family members at risk is recommended by measuring tHcy but molecular genetic testing may also be utilized in exceptional cases (grade D).

Monitoring of tHcy, amino acids, folate and vitamin B12 is recommended in all patients during therapy. The frequency of the monitoring is variable on a case-by-case basis (due to severity, treatment plan, age,

etc). The targeted concentration ranges for total plasma homocysteine are proposed to be <50 µmol/L in pyridoxine-responsive patients and at <11 µmol/L free homocysteine (about 120 µmol/L total homocysteine) in pyridoxine-unresponsive patients (Morris et al., 2017).

Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

On May 13, 2011, the FDA approved the Invader *MTHFR* 677 created by Hologic, Inc. The Invader *MTHFR* 677 is an in-vitro diagnostic test intended for the detection and genotyping of a single point mutation (C to T at position 677) of the human 5,10-methylenetetrahydrofolate reductase (*MTHFR*) gene in isolated genomic DNA obtained from whole blood Potassium EDTA samples from patients with suspected thrombophilia (FDA, 2011a).

On April 25, 2011, the FDA approved the Invader *MTHFR* 1298 created by Hologic, Inc. The Invader *MTHFR* 1298 test is an in vitro diagnostic test intended for the detection and genotyping of a single point mutation (A to C at position 1298) of the human 5,10-methylenetetrahydrofo late reductase (*MTHFR*) gene in isolated genomic DNA obtained from whole blood potassium EDTA samples from patients with suspected thrombophilia (FDA, 2011b).

On April 22, 2010, the FDA approved the eSensor Thrombophilia Risk Test on XT-8 System created by Osmetech Molecular Diagnostics. The *MTHFR*-specific portion is as follows: The eSensor *MTHFR* Genotyping Test is an in-vitro diagnostic for the detection and genotyping of point mutations (C to T at position 677) and (A to C at position 1298) of the human 5,10-methylenetetrahydrofo late reductase (*MTHFR*) gene in isolated genomic DNA obtained from whole blood samples. The test is intended to be used on the eSensor XT-8 System (FDA, 2010).

On October 11, 2007, the FDA approved the Verigene System created by Nanosphere Inc. The *MTHFR*-specific portion is as follows: The Verigene *MTHFR* Nucleic Acid Test is an in vitro diagnostic for the detection and genotyping of a single point mutation (C to T at position 677) of the human 5,10-methylenetetrahydrofolate reductase gene (*MTHFR*) in patients with suspected thrombophilia, from isolated genomic DNA obtained from whole blood samples. The test is intended to be used on the Verigene System (FDA, 2007).

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
82136	Amino acids, 2 to 5 amino acids, quantitative, each specimen
82139	Amino acids, 6 or more amino acids, quantitative, each specimen
82615	Cystine and homocysteine, urine, qualitative
83090	Homocysteine
83921	Organic acid, single, quantitative
84207	Pyridoxal phosphate (Vitamin B-6)

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

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Revision History

Revision Date	Summary of Changes
12/04/2024	Reviewed and Updated: Updated the background, guidelines and recommendations, and evidence-based scientific references. Literature review did not necessitate any modifications to coverage criteria.

Therapeutic Drug Monitoring for 5-Fluorouracil

Policy Number: AHS – M2067 – Therapeutic Drug Monitoring for 5-Fluorouracil	Initial Presentation Date: 09/18/2015 Effective Date: 4/1/2025

Policy Description

Chemotherapeutic agents are incredibly potent drugs, often carrying cytotoxic side effects. Most chemotherapeutic drugs have a steep dose-response relationship and a narrow therapeutic index (a range where an agent provides therapeutic effect without major side effects). Identification of the optimal dose of a chemotherapeutic agent, such as 5-fluorouracil, has been proposed as a potential improvement for the management of cancer patients (Eaton, 2024).

This policy does not address pharmacogenetic testing to aid or direct chemotherapies. For pharmacogenetic testing, please refer to AHS-M2021.

Related Policies

Policy Number	Policy Title
AHS-M2021	Pharmacogenetic Testing

Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) For individuals who are undergoing 5-fluorouracil chemotherapy, therapeutic drug monitoring (TDM) to aid in managing dose adjustment **MEETS COVERAGE CRITERIA**.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

2) To aid in managing dose adjustment for individuals undergoing 5-fluorouracil chemotherapy, the following tests **DO NOT MEET COVERAGE CRITERIA**:
   a) Uracil breath tests.
   b) Dihydrouracil/uracil ratio testing of plasma, serum, or urine samples.

Table of Terminology

Term	Definition
5-FU	5-fluorouracil
AUC	Area-under-curve
BSA	Body surface area
CCYR	Complete cytogenetic response
CHEERS	Consolidated Health Economic Evaluation Reporting Standards
CLIA	Clinical Laboratory Improvement Amendments of 1988
CMS	Centers for Medicare and Medicaid
CPIC	Clinical Pharmacogenetics Implementation Consortium
CRCL	Creatinine clearance
DPD/DPYD	Dihydropyrimidine dehydrogenase
FU	Fluorouracil
GFR	Glomerular filtration rate
GPCO	Groupe de Pharmacologie Cinique Oncologique
HPLC	High-Performance Liquid Chromatography
IATDMCT	International Association of Therapeutic Drug Monitoring and Clinical Toxicology
LC-MS/MS	Liquid Chromatography-Tandem Mass Spectrometry
LDT	Laboratory-developed tests
MMR	Major molecular response
NCCN	National Comprehensive Cancer Network
NICE	National Institute for Health and Care Excellence
OS	Overall survival
PK	Pharmacokinetic
RCT	Randomized control trials
SCCHYN	Squamous cell carcinoma of the head and neck
SFPT	Group of The French Society of Pharmacology and Therapeutics
STP-PT	Therapeutic Pharmacological Monitoring and Personalization of Treatments
TDM	Therapeutic drug monitoring
TOPS	Tyrosine kinase inhibitor optimization and selectivity
TYMS	Thymidylate synthase

Scientific Background

Chemotherapeutic agents encompass a wide variety of medications used to treat cancer. However, due to their cytotoxicity, these agents often have debilitating side effects such as nausea, vomiting, and more. Therefore, it can be useful to identify an "optimal" dose of these agents (for an individual patient) maximize therapeutic efficacy and minimize harmful side effects. Numerous methods to identify an individual's optimal dose exist, such as body surface area (BSA)-based dosing, weight-based dosing, fixed-dose medications, and area-under-curve (AUC) dosing, which is generated by a curve of plasma concentration as a function of time. With both variables known, it would be possible to identify the exact amount of drug exposed to an individual instead of relying on clinical symptoms. AUC-based dosing is typically used for drugs cleared through glomerular filtration (such as carboplatin). However, AUC-based dosing is not usually applicable to most other anticancer agents as elimination of other drugs often involves several other pathways, thereby introducing additional variables that influence drug clearance (Eaton, 2024).

One common therapeutic agent is 5-fluorouracil, or 5-FU. Currently, 5-FU is administered intravenously as a continuous infusion; BSA-based dosage is often used to optimize treatment, and an AUC between 20 and 30 [mg×h×L] is recommended (Mindt et al., 2019). This particular chemotherapeutic agent can be used alone, or in a combinatory setting, to treat many types of cancer including breast, anal, stomach, colon, head, neck, and some skin cancers (Cancer Research, 2024). Therapeutic drug monitoring (TDM), known as "the clinical practice of measuring specific drugs at designated intervals to maintain a constant concentration in a patient's bloodstream, thereby optimizing individual dosage regimens" (Kang & Lee, 2009), has shown promise in 5-FU based treatment regimens. In particular, the TDM practice has resulted in reduced toxicity and improved efficacy for the intravenous administration of 5-FU (Hashimoto et al., 2020).

Proprietary Testing

Proprietary tests have been developed for identification of the optimal dose of several chemotherapeutic agents. Saladax Biomedical, under the product umbrella termed MyCare, offers a series of tests that aim to find the optimal dose for various chemotherapeutic agents. Their current catalog includes tests for 5-FU (My5-FU), paclitaxel (MyPaclitaxel), docetaxel (MyDocetaxel), and imatinib (MyImatinib). MyCare states that these tests will be able to guide dosing for these agents and minimize toxicity with only a blood test (MyCare, 2024a, 2024b). The test is intended for patients receiving 5-FU chemotherapy through intravenous infusion. The test takes plasma near the end of the infusion cycle and is based on the scattered light principle. The amount of scattered light varies inversely with the amount of 5-FU present in the plasma sample. The limit of detection is estimated at 52 ng/mL and the limit of quantitation is estimated at 85 ng/mL. A validated dose adjustment algorithm incorporates the measurements of 5-FU in plasma and uses AUC to calculate subsequent doses (NICE, 2014).

Additional tests have been proposed to aid in dosing and measuring toxicity in individuals undergoing chemotherapy. Since the efficacy of 5-FU depends on the enzyme dihydropyrimidine dehydrogenase (DPD), the concentration of uracil has been proposed to evaluate pyrimidine, including 5-FU, catabolism. The uracil breath test measures the concentration of carbon dioxide, a pyrimidine metabolic product, after an individual has ingested radiolabeled uracil (Cunha-Junior et al., 2013; Ezzeldin et al., 2009).

Analytical Validity

Buchel et al. (2013) compared My5-FU to other commonly used clinical analyzers (Olympus AU400, Roche Cobas c6000, and Thermo Fisher CDx90). A total of 247 plasma samples were measured. The Cobas Integra 800 was found to have a "proportional bias of 7% towards higher values measured with the My5-FU assay" compared to liquid chromatography-tandem mass spectrometry (LC-MS/MS). However, when Cobas Integra 800 was compared to the other three clinical analyzers, only a proportional bias of ≤1.6% and a constant bias below the limit of detection was observed (Buchel et al., 2013).

Clinical Utility and Validity

Yang et al. (2016) conducted a meta-analysis of data from two randomized control trials (RCTs) and three observational studies (654 patients) to compare the efficacy and toxicity of the use of pharmacokinetic (PK)-guided versus Body Surface Area (BSA)-based dose adjustment of 5-FU in advanced cancers. PK-monitored 5-FU therapy was found to be associated with "significant improvement in overall response rate (odds ratio = 2.04) compared with the traditional BSA method." The researchers concluded that "in comparison with conventional BSA method, PK-based 5-FU dosage confirmed a superior overall response rate and improved toxicities irrespective of significant difference, the results of which indicated that PK- monitored 5-FU dosage has the potential to be performed in colorectal cancer personalized therapy" (Yang et al., 2016).

Fang et al. (2016) performed a meta-analysis to compare the BSA-based algorithm to a pharmacokinetic (PKG)-based algorithm for 5-fluorouracil (5-FU). Four studies (n = 504) were included. The authors found that the PKG algorithm "significantly" improved the objective response rate of 5-FU chemotherapy compared to the BSA-based algorithm. PKG was also found to "markedly" decrease the risk of grade 3/4 adverse drug reactions (Fang et al., 2016). Likewise, another study comparing 5-FU TDM to BSA-guided dosing results in patients with gastrointestinal cancer (n = 155) also reports greater interpersonal variability when using a BSA-guided strategy as compared to TDM (Morawska et al., 2018). A third study demonstrates that TDM can result in even greater improvements in elderly gastrointestinal cancer patients (older than 75 years old) as compared to younger patients (71% improvement in AUC vs. 50% improvement, respectively). This is significant considering that the majority of previous clinical trials excluded elderly patients (Macaire et al., 2019).

Wilhelm et al. (2016) evaluated the use of TDM to personalize 5-FU dosing in patients with colorectal cancer. Seventy-five patients were included. The authors aimed to achieve a target AUC of 20-30 mg x h/L and adjusted each cycle of 5-FU accordingly. The average AUC of 5-FU on the initial administration was "18 ± 6 mg × h/L, with 64%, 33%, and 3% of the patients below, within, or above the target AUC range, respectively." By the fourth administration, the average 5-FU AUC was 25 ± 7 mg × h/L, with 54% of patients within the target 5-FU AUC range. The incidence of 5-FU related side effects was reduced compared to historical data despite the increased dose. The authors concluded that "personalization of 5-FU dosing using TDM in routine clinical practice resulted in significantly improved 5-FU exposure and suggested a lower incidence of 5-FU-related toxicities" (Wilhelm et al., 2016).

Gamelin et al. (2008) conducted a study to compare conventional dosing of fluorouracil (FU) with pharmacokinetically guided FU dose adjustment in terms of response, tolerability, and survival. A total of 208 patients with measurable metastatic colorectal cancer were randomly assigned to two groups: group A (104 patients; 96 assessable), in which the FU dose was calculated based on body-surface area; and group B (104 patients; 90 assessable), in which the FU dose was individually determined using pharmacokinetically guided adjustments. Patients that received FU dose adjustment based on pharmacokinetic monitoring showed significantly improved objective response rate, a trend to higher

survival rate, and fewer grade 3/4 toxicities. The researchers concluded that "these results support the value of pharmacokinetically guided management of FU dose in the treatment of metastatic colorectal patients" (Gamelin et al., 2008).

Engels et al. (2011) examined the effect of pharmacokinetic (PK)-guided docetaxel dosing on interindividual variability in exposure. AUC was used to guide dosing, and 15 patients were included. The authors found that variability (standard deviation) decreased by 35% after one course of PK-guided dosing. However, the authors stated further research was needed (Engels et al., 2011).

Joerger et al. (2007) built a pharmacokinetic-pharmacodynamic model of paclitaxel/carboplatin in ovarian cancer patients. Time above paclitaxel plasma concentration of 0.05 to 0.2 µmol/L (t_c> 0.05−0.2 µmol/L) is thought to be a good predictive marker for severe neutropenia and overall clinical outcome. A total of 139 patients were included in the study; each participant was given "175 mg/m2 over 3 hours followed by carboplatin area under the concentration-time curve 5 mg/mL*min over 30 min." In 34 patients with measurable disease, objective response rate was 76%. Paclitaxel t_c > 0.05 µmol/L was found to be significantly higher in patients with a complete (t = 91.8 hours) or partial response (t = 76.3) compared to patients with progressive disease (t = 31.5). Paclitaxel t_c was also found to predict severe neutropenia well (Joerger et al., 2007).

A 2017 study by Moeung et al. (2017) evaluated the efficacy of TDM in patients (n = 89) with advanced germ cell tumors who receive high dose chemotherapy (TI-CE) as compared to using a formula-based covariate equation dosing method. The metric used to assess the efficacy of these two approaches was AUC for carboplatin. TDM was used on 58 of the patients for three days "to develop a covariate equation for carboplatin clearance prediction adapted for future TI-CE patients, and its performance was prospectively evaluated on the other 29 patients along with different methods of carboplatin clearance prediction." Using the developed covariate equation to determine dosing, the researchers showed that the mean AUC was 24.4 mg.min/ml per cycle with 10^{th} and 90^{th} percentiles of 22.4 and 26.8, respectively. They conclude, "TDM allows controlling and reaching the target AUC." An alternative is using "the new equation of carboplatin clearance prediction," a strategy better adapted for young individual patients when TDM cannot be used (Moeung et al., 2017). However, more recent studies have also shown that the method to determine carboplatin clearance (for example, glomerular filtration rate (GFR) versus estimated creatinine clearance (CrCl)) can have a significant effect on determining the actual AUC for carboplatin (Morrow et al., 2019).

Guilhot et al. (2012) evaluated the correlation between "imatinib trough plasma concentrations (C_{min}) and clinical response and safety in patients with newly diagnosed Philadelphia chromosome-positive chronic myeloid leukemia in chronic phase in the Tyrosine Kinase Inhibitor OPtimization and Selectivity (TOPS) trial." Patients were randomized to 400 mg/day or 800 mg/day of imatinib. The authors found that the C_{min} was stable for patients in the 400 mg/day cohort but showed a slight decrease in the 800 mg/day cohort due to dose adjustments. The rates of major molecular response (MMR) and complete cytogenetic response (CCyR) was found to be significantly lower in patients under the twenty fifth percentile of C_{min} (1165 ng/mL). The authors also observed an association between high imatinib C_{min} and side effects such as edema (Guilhot et al., 2012).

Freeman et al. (2015) evaluated the clinical and cost effectiveness of the My5-FU assay. The authors compared the assay to gold standards of serum testing and chemotherapeutic dosing. Thirty-five studies regarding clinical effectiveness and 54 studies regarding cost effectiveness were identified. The investigators identified a high "apparent" correlation between My5-FU, high-performance liquid chromatography (HPLC), and liquid chromatography-mass spectrometry (LC-MS), although upper and

lower limits of agreement ranged from -18% and 30%. Median overall survival (OS) was found to be 19.6 months for pharmacokinetic dosing (PK) compared to 14.6 months for body surface area (BSA)-guided dosing of 5-FU plus folinic acid. The authors also built a cost-effectiveness model for the My5-FU assay for metastatic colorectal cancer and head and neck cancer. The model showed My5-FU to be 100% cost effective at £20,000 per quality-adjusted life-year for both types, although the head and neck cancer was only an estimate. Despite these findings, the authors noted that "considerable uncertainties remain about evidence quality and practical implementation" (Freeman et al., 2015).

Cunha-Junior et al. (2013) studied the use of the uracil breath test to determine 5-FU toxicity in gastrointestinal cancer patients (n = 33). Their results show that the uracil breath test had a sensitivity and specificity of 61.5% and 85%, respectively in distinguishing individuals with grade 3-4 versus grade 0-1 toxicity. Likewise, the sensitivity and specificity of distinguishing DPD-deficiency versus non-DPD-deficiency are 75% and 85%, respectively. The authors conclude that the uracil breath test "has moderate accuracy in discriminating individuals who manifested severe toxicity from those who had mild or no toxicity to 5FU" (Cunha-Junior et al., 2013).

Macaire et al. (2019) researched the effects of TDM to optimize 5-FU chemotherapy in gastrointestinal cancer patients under and over 75 years of age. A total of 154 participants with gastrointestinal cancer participated in this study; thirty-one participants were older than 75 years of age. "At cycle 1 (C1), the 5-FU dose was calculated using patient's body surface area, then a blood sample was drawn to measure 5-FU concentration and 5-FU dose was adjusted at the subsequent cycles based on C1 concentration. Assessments of toxicity were performed at the beginning of every cycle" (Macaire et al., 2019). Results show that approximately 71% of patients older than 75 years of age required dose adjustments after C1, while only 50% of younger patients required adjustments. Further, after dose adjustments, by cycle 3 (C3), the percentage of patients above age 75 with severe 5-FU related toxicity fell from 15% to 5%. The authors conclude that "Pharmacokinetic-guided 5-FU-dosing algorithm, leading to an improved tolerability while remaining within therapeutic concentration range, is even more valuable for patients older than 75 years than in younger patients" (Macaire et al., 2019).

Deng et al. (2020) studied the efficacy of pharmacokinetic-based 5-FU dosing management in advanced colorectal cancer patients. A total of 153 patients with advanced colorectal cancer were randomized to receive a double-week chemotherapy with 5-FU using pharmacokinetic dosing or 5-FU chemotherapy with BSA guided dosing. In the first four weeks of treatment, patients in the experimental group were administered 5-FU according to the classic strategy of body surface area dosing before transitioning into pharmacokinetic AUC-based dosing. For the duration of the study, all patients in the control group continued with BSA guided chemotherapy. The efficacy, toxic side effects, and survival rate were assessed throughout the study. In the AUC-based dosing (experimental) group, "the rate of diarrhea significantly decreased (37.50% vs. 70.00%, P=0.010), and incidence of oral mucositis reduced (54.17% vs. 82.50%, P=0.014). Compared with the control group, the clinical benefit rate of experimental group was much higher (90.79% vs. 79.22%, P=0.046)." There was no significant difference in other 5-FU related toxic side effects such as nausea or vomiting and no difference in progression-free survival between the two groups. The authors concluded that "pharmacokinetic- based dose management of 5-Fluorouracil reduces the toxicity of chemotherapy and improves long-term efficacy of chemotherapy for advanced colorectal cancer patients" (Deng et al., 2020).

Dolat et al. (2020) studied how evaluating DPD deficiency before initiating 5-FU treatment could help limit 5-FU toxicity by investigating the relationship between 5-GU clearance and DPD activity markers. There were 169 patients with colorectal, pancreas, and metastatic cancer included in the study and the DPD marker, uracilemia (U), was measured. Overall, all patients benefited from a pre-therapeutic DPYD

genotyping and phenotyping. There was no correlation between uracilemia levels and 5-FU clearance. However, in patients with low DPD marker levels (U<16 ng/mL), 5-FU exposure was higher than in other patients and these patients benefited from an increase in dose following 5-FU therapeutic drug monitoring (TDM). The author states that if guidelines recommend decreasing the 5-FU dose in patients with U > 16 ng/mL, then these patients are at risk of under-exposure and 5-FU TDM should be conducted to avoid loss of efficacy (Dolat et al., 2020).

Vithanachchi et al. (2021) reviewed the economic evaluations of TDM interventions for certain cancer drugs. Through identifying 11 publications, the researchers found that TDM with imatinib and TDM with 5-FU were the "most commonly assessed interventions." Using the Consolidated Health Economic Evaluation Reporting Standards (CHEERS) Checklist, they evaluated the quality of reporting of economic evaluations, and found that these publications met 61-91% of CHEERS checklist criteria. Additionally, "all publications considered TDM to be cost-effective based on an incremental cost-effectiveness ratio below the willingness to pay threshold (64%) or being cost-saving (36%)," and TDM interventions were likely to be "cost-effective in an oncology landscape where treatments offering small benefits have high cost." To fully evaluate the impact of TDM, the researchers also suggest assessing uncertainties in the clinical evidence for newer treatments used alongside or after TDM treatment. This research elucidated the context by which TDM could be beneficial fiscally and how that may impact future care.

Laures et al. (2022) investigated DPD deficiency screening using uracil-based phenotyping to see whether it reduced the negative side effects of 5-Fluorouracil-based chemotherapy. French recommendations call for screening for DPD deficiency (through plasma uracil quantification) before instituting fluoropyrimidine-based chemotherapy. A total of 198 patients who received 5-FU therapy (these participants had DPD deficiency) were compared to 94 reference patients. According to the authors, the study showed a reduction in 5-FU serious toxic events during the first four courses of chemotherapy. Their analysis "identified a significant difference in adverse effects toxicity coupled with their frequency between patients with an identified DPD phenotype and patients with an unknown DPD phenotype." However, the authors also described how various studies of DPD deficiency have given conflicting results. For example, a separate study "demonstrated no significant difference in the prevalence of toxicities between DPD-deficient and non-deficient patients, suggesting that further work is needed to investigate the association of phenotyping with toxicity" (Laures et al., 2022; Tejedor-Tejada et al., 2022)

Guidelines and Recommendations

International Association of Therapeutic Drug Monitoring and Clinical Toxicology (IATDMCT)

The IATDMCT released guidelines on the dosing of 5-FU. With regards to assessing systemic exposure to 5-FU, the IATDMCT noted that area-under-curve (AUC) was the "accepted and clinically relevant" metric. They also noted that a relationship existed between 5-FU AUC and clinical activity (as well as toxicity. They go on to state, "It should be noted that statistically significant correlations between 5-FU exposure and toxicity have been observed across several disease types (squamous cell carcinoma of the head and neck (SCCHYN), nasopharyngeal cancer, and CRC), disease settings (metastatic, locally advanced), and dosing types (bolus, infusion)." Also, they note that "several clinical studies…have found statistically significant correlations between 5-FU exposure and clinical outcome, mostly with response rates being the metric, but also indicated by overall survival" (Beumer et al., 2019; NICE, 2014).

The IATDMCT also made remarks on the use of TDM for 5-FU. They noted that TDM reduced variability and toxicity, as well as improved clinical activity in patients receiving 5-FU, and "strongly recommend"

TDM for the management of 5-FU therapy in patients with colorectal or head-and-neck cancer receiving common 5-FU regimens (Beumer et al., 2019).

Concerning the use of the uracil breath test, the IATDMCT states, "The uracil breath test does not help in determining the correct does and is not recommended for clinical use" (Beumer et al., 2019).

National Comprehensive Cancer Network (NCCN)

The NCCN published guidelines on management of antiemesis, intended to control one of chemotherapy's primary side effects. In it, the only chemotherapeutic agent listed with an AUC-based dosing regimen is carboplatin. Docetaxel, 5-FU and paclitaxel are listed as having 10-30% emetic risk whereas imatinib <=400 mg/day is listed as <30% risk. No information regarding therapeutic drug monitoring was included (NCCN, 2024a). Furthermore, the NCCN did not address TDM in either its colon cancer or head and neck cancer guidelines (NCCN, 2024b, 2024c).

National Institute for Health and Care Excellence (NICE)

The NICE remarked that the My5-FU assay should only be recommended for research purposes, although they noted that it has "promise" (NICE, 2014). In a December 2017 review of the 2014 guideline, NICE stated that no changes were required (NICE, 2017).

Clinical Pharmacogenetics Implementation Consortium (CPIC)

In 2017, the CPIC published updated guidance on dihydropyrimidine dehydrogenase (*DPYD*) genotyping and fluoropyrimidine (5-FU) dosing. The following recommendations are related to TDM:

- "In *DPYD* poor metabolizers (DPYD-AS: 0.5 or 0), it is strongly recommended to avoid use of 5-fluorouracil containing regimens. However, if no fluoropyrimidine-free regimens are considered a suitable therapeutic option, 5-fluorouracil administration at a strongly reduced dose combined with early therapeutic drug monitoring may be considered for patients with *DPYD*-AS of 0.5. It should be noted, however, that no reports of the successful administration of low dose 5-fluorouracil in *DPYD* poor metabolizers are available to date."
- "Pharmacokinetically-guided dosing of 5-fluorouracil has been shown to result in an increase in the proportion of patients with 5-fluorouracil exposure (AUC) within the targeted therapeutic range and a reduced number of 5-fluorouracil related adverse effects. In particular, to avoid underdosing of patients with genotype-based dose reductions who tolerate higher 5-fluorouracil doses, follow-up therapeutic drug monitoring is recommended."
- For *DPYD* intermediate metabolizers, the following dosing recommendation was given: "Reduce starting dose based on activity score followed by titration of dose based on toxicity or therapeutic drug monitoring (if available)."
- For *DPYD* poor metabolizers, the following dosing recommendation was given: "In the event, based on clinical advice, alternative agents are not considered a suitable therapeutic option, 5-fluorouracil should be administered at a strongly reduced dosed with early therapeutic drug monitoring" (Amstutz et al., 2018).

Therapeutic Pharmacological Monitoring and Personalization of Treatments (STP-PT) Group of The French Society of Pharmacology and Therapeutics (SFPT) and the Groupe de Pharmacologie Cinique Oncologique (GPCO)

The STP-PT group of the SFPT and GPCO on 5-FU therapeutic drug monitoring state that "based on the latest and most up-to-date literature data, [we] recommend the implementation of 5-FU Therapeutic Drug Monitoring in order to ensure an adequate 5-FU exposure" (Lemaitre et al., 2018).

Francophone Network of Pharmacogenetics (RNPGx) and the French Clinical Oncopharmacology Group (GPCO)-UNICANCER

Etienne-Grimaldi et al. (2023) released "Current diagnostic and clinical issues of screening for dihydropyrimidine dehydrogenase deficiency [DPD]," which included recommendations for FP-based chemotherapy. The guideline recommends the following:

- "EMA recommends DPD testing (*DPYD* variants or uracilemia) before FP-based chemotherapy.
- Genotyping relevance of the 4 consensual *DPYD* variants is restricted to Caucasians.
- *DPYD* genotype-guided FP dose reduction is clinically validated, contrary to uracilemia.
- Impact of DPD-guided FP dose reduction on efficacy needs further investigation.
- 5FU therapeutic drug monitoring is recommended in partial DPD-deficient patients" (Etienne-Grimaldi et al., 2023).

Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

The FDA's "Prescribing Information" documents for fluorouracil, paclitaxel, imatinib, and docetaxel do not include AUC as a method to adjust dosage (FDA, 2016a, 2016b, 2018, 2021).

Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
S3722	Dose optimization by area under the curve (AUC) analysis, for infusional 5-fluorouracil
80299	Quantitation of therapeutic drug, not elsewhere specified

82542	Column chromatography, includes mass spectrometry, if performed (eg, HPLC, LC, LC/MS, LC/MS-MS, GC, GC/MS-MS, GC/MS, HPLC/MS), non-drug analyte(s) not elsewhere specified, qualitative or quantitative, each specimen
83789	Mass spectrometry and tandem mass spectrometry (eg, MS, MS/MS, MALDI, MS-TOF, QTOF), non-drug analyte(s) not elsewhere specified, qualitative or quantitative, each specimen

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

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Revision History

Revision Date	Summary of Changes
12/04/2024	Reviewed and Updated: Updated background, guidelines, and evidence-based scientific references. Literature review did not necessitate any modification to the coverage criteria.

Testosterone

Policy Number: AHS – G2013 – Testosterone	Initial Presentation Date: 11/16/2015 Effective Date: 4/1/2025

Policy Description

Testosterone is a naturally occurring lipophilic androgen hormone that is produced by both males and females for various functions. In males, testosterone is produced by the interstitial cells of Leydig in the testis. In females, testosterone is primarily created and disseminated by the ovaries and adrenal glands. Testosterone is required for synthesis of dihydrotestosterone (DHT) as well as estradiol (E2). Sex hormone-binding globulin (SHBG) binds testosterone to aid in transport and intratesticular bioavailability.

Dysregulation in testosterone levels can lead to serious conditions, including hypogonadism and other testosterone excess or deficiency conditions. Additional hormones, including follicle-stimulating hormone (FSH), luteinizing hormone (LH), and prolactin, play roles in development. As part of the hypothalamic-pituitary-gonadal axis, FSH and LH bind to gonadal receptors to modulate testosterone. During conditions of dyshomeostasis, such as hypogonadism, FSH, LH, and prolactin serum levels can be used as diagnostic tools (Bhasin et al., 2018; Gill-Sharma, 2018).

Terms such as male and female are used when necessary to refer to sex assigned at birth.

Related Policies

Policy Number	Policy Title
N/A	Not Applicable

Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) Measurement of serum total testosterone (see Note 1) **MEETS COVERAGE CRITERIA** in **any** of the following situations:
    a) For symptoms of androgen deficiency or androgen excess in males:
        i) For initial screening, two measurements at least 24 hours apart.
        ii) If the initial screening was normal but symptoms persist, follow-up testing is allowed no sooner than 60 days after the initial screening.
    b) For the monitoring of treatment response in men taking enzyme inhibitors for prostate cancer.
    c) For men receiving testosterone replacement therapy (every 2-3 months for the first year after initiation of therapy or after a change in therapeutic dosage; annually thereafter).
    d) For gender-dysphoric/gender-incongruent persons (baseline, during treatment, and for therapy monitoring).
    e) For symptomatic females (see Note 2) being evaluated for conditions associated with androgen excess (e.g., polycystic ovary syndrome and functional hypothalamic amenorrhea).

2) For males with total testosterone confirmed as low or borderline low **and** who have hypogonadism, gynecomastia, and/or other forms of testicular hypofunction, annual measurement of serum free testosterone, sex hormone-binding globulin (SHBG), and/or albumin **MEETS COVERAGE CRITERIA**.

3) For individuals suspected of having a disorder that is accompanied by increased or decreased SHBG levels (see Notes 3 and 4), measurement of serum free testosterone using a medically accepted algorithm based on total serum testosterone, SHBG, and/or albumin or bioavailable testosterone **MEETS COVERAGE CRITERIA**.

4) Prior to initiating testosterone therapy for males with gynecomastia, once per lifetime serum estradiol measurement **MEETS COVERAGE CRITERIA**.

5) For individuals with ambiguous genitalia, hypospadias, or microphallus, measurement of serum dihydrotestosterone for the diagnosis of 5-alpha reductase deficiency **MEETS COVERAGE CRITERIA.**

6) Measurement of serum free testosterone and/or bioavailable testosterone as a primary test (i.e., in the absence of prior serum total testosterone measurement) **DOES NOT MEET COVERAGE CRITERIA**.

7) For asymptomatic individuals or for individuals with non-specific symptoms, measurement of serum total testosterone, free testosterone, and/or bioavailable testosterone **DOES NOT MEET COVERAGE CRITERIA**.

8) For the identification of androgen deficiency in women, measurement of serum testosterone **DOES NOT MEET COVERAGE CRITERIA**.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

9) The use of saliva for the measurement of testosterone **DOES NOT MEET COVERAGE CRITERIA**.

10) For all other situations not mentioned above, measurement of serum dihydrotestosterone **DOES NOT MEET COVERAGE CRITERIA**.

NOTES:

Note 1: Serum total testosterone sample collection should occur in the early morning, after fasting. Due to considerable variability in serum total testosterone levels, the Centers for Disease Control and Prevention (CDC) developed a standardization program for total testosterone assays (Hormone Standardization [HoSt]/Testosterone). An assay certified by the CDC's HoSt/Testosterone program is standardized to within ±6.4% of the CDC total testosterone reference standard. It is **STRONGLY RECOMMENDED** that serum total testosterone measurement be performed with an assay that has been certified by the CDC HoSt/Testosterone program (Bhasin et al., 2018). A list of CDC-certified assays is available on the HoSt website (CDC, 2023).

Note 2: When measuring serum total testosterone in females, please note that the technology used for measurement must be sensitive enough to detect the low serum total testosterone levels that are normally found in females.

Note 3: Conditions associated with decreased SHBG concentrations according to the 2018 Endocrine Society Guidelines (Bhasin et al., 2018):

- Obesity
- Diabetes mellitus
- Use of glucocorticoids, progestins, and androgenic steroids
- Nephrotic syndrome
- Hypothyroidism
- Acromegaly
- Polymorphisms in the SHBG gene

Note 4: Conditions associated with increased SHBG concentrations according to the 2018 Endocrine Society Guidelines (Bhasin et al., 2018):

- Aging
- HIV disease
- Cirrhosis and hepatitis
- Hyperthyroidism
- Use of some anticonvulsants
- Use of estrogens
- Polymorphisms in the SHBG gene

Table of Terminology

Term	Definition

AAP	American Academy of Pediatrics
ABIM	American Board of Internal Medicine
ABP	Androgen binding protein
ACOG	The American College of Obstetricians and Gynecologists
ACTH	Adrenocorticotropic hormone
ADT	Androgen deprivation therapy
AFP	Alpha- fetal protein
ASRM	American Society of Reproductive Medicine
AIDS	Acquired immunodeficiency syndrome
AUA	American Urological Association
CBG	Corticosteroid-binding globulin-bound testosterone
CDC	Centers for Disease Control and Prevention
CLIA'88	Clinical Laboratory Improvement Amendments of 1988
CMAJ	Canadian Medical Association Journal
CMS	Centers for Medicare and Medicaid
CSAM	Canadian Society of Endocrinology and Metabolism
CUA	Canadian Urological Association
CV	Coefficient of variation
DHEAS	Dehydroepianandrosterone sulphate
DHT	Dihydrotestosterone
E2	Estradiol
EAA	European Academy of Andrology
EAU	European Association of Urology
ELISA	Enzyme linked immunosorbent assay
ES	The Endocrine Society
ESI	Electrospray ionization
FDA	Food and Drug Administration
FHA	Functional hypothalamic amenorrhea
FSH	Follicle-stimulating hormone
FT	Free testosterone
Gy	Gray unit of ionizing radiation
hCG	Human chorionic gonadotropin
HIV	Human immunodeficiency virus
HoSt	Hormone standardization
HPLC	High performance liquid chromatography
ID	Isotope dilution
ID-LC-MS	Isotope dilution-liquid chromatography-tandem mass spectrometry
IGHG	International Late Effects of Childhood Cancer Guideline Harmonization Group
LC-MS/MS	Liquid chromatography-tandem mass spectrometry
LDTs	Laboratory-developed tests
LH	Luteinizing hormone
LOH	Late onset hypogonadism
MRI	Magnetic resonance imaging

MS	Mass spectrometry
NCCN	National Comprehensive Cancer Network
OH	Hydroxy
ORM	Orosomucoid bound testosterone
PADAM	Partial androgen deficiency
PCOS	Polycystic ovary syndrome
PCSF	PanCareSurFup
PRL	Prolactin
PSA	Prostatic specific antigen
SHBG	Sex hormone binding globulin
SHBG	Sex hormone binding globulin-bound testosterone
TBI	Traumatic Brain Injury
TBS	Trabecular bone score
THS	Tetrahydrocortisol
TSH	Thyroid stimulating hormone
TT	Total testosterone
vBMD	Volumetric bone mineral density
WC	Waist circumference

Scientific Background

The steroid hormone, testosterone, plays a role in both male and female development and health. In males, testosterone is involved in the stage-specific differentiation of germ cells, spermatogenesis, and the synthesis of dihydrotestosterone (DHT) and estradiol (E2). DHT stimulates sexual differentiation of male genitalia during embryogenesis, genital maturation during puberty, and growth of pubic and facial hair (Kinter & Anekar, 2020). E2 is required in males for modulating libido, erectile function, and spermatogenesis (Schulster et al., 2016). Serum testosterone is typically solubilized by binding to the androgen-binding protein (ABP) or SHBG, which aids in regulating their transport, distribution, metabolism, and biological activity. ABP and SHBG have similar primary structure, but they differ in the types of oligosaccharides associated with them (Hammond & Bocchinfuso, 1995).

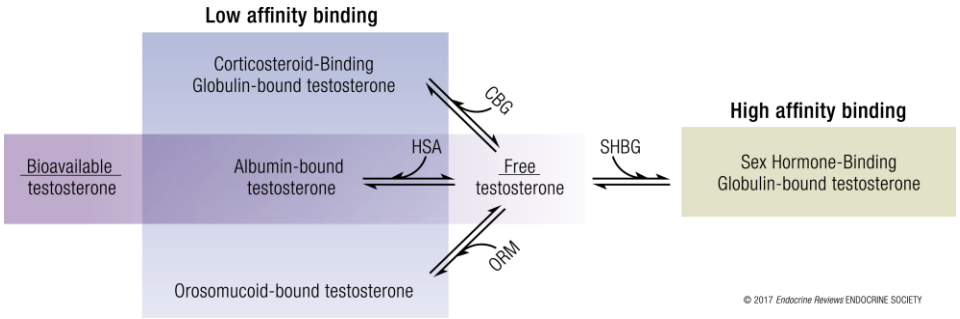
In females, testosterone is primarily synthesized and secreted in the ovaries and adrenal glands (Longcope, 1986) but some testosterone production also occurs in peripheral tissues like muscle, fat, breast, and bone (Burger, 2002). Polycystic ovary syndrome (PCOS) is one manifestation of a dysregulation of testosterone in women and is a complicated condition with a variety of metabolic, reproductive, and psychological features (Teede et al., 2018).

Primary and secondary hypogonadism are two forms of testicular hypofunction found in males. These conditions can be differentiated by the concentration of serum LH, FSH, and prolactin. Primary hypogonadism is associated with low levels of testosterone and normal to high levels of LH and FSH. Secondary hypogonadism is associated with low levels of testosterone and normal to low levels of LH and FSH (Carnegie, 2004). The anterior pituitary gland of hypothalamic-pituitary-gonadal axis releases LH and FSH, which act on the gonadal receptors to regulate testosterone production. Binding of LH to Leydig cell receptors initiates testosterone production, while testosterone secretion is further regulated by feedback inhibition (Nassar & Leslie, 2023). Males who develop hypogonadism prior to puberty often exhibit depressed secondary sex characteristics, eunuchoid stature, small testes, gynecomastia, and a small phallus. For males who develop hypogonadism after the onset of puberty, the physical findings are

similar, except for a normal stature and normal phallus size (Snyder, 2024). Besides hypogonadism, testosterone production can also be affected by certain medications, chemotherapy, lifestyle, and aging (Meldrum et al., 2012; Nassar & Leslie, 2023).

In adult males, total serum testosterone levels decrease at an average rate of 1.6% per year. The concentrations of free and bioavailable testosterone decrease more rapidly, typically two to three percent annually, due to the natural increase in SHBG. By the age of 60, 20% of men will have testosterone levels below the normal range, "and the figure rises to 50% in those aged over 80" (Stanworth & Jones, 2008). Significant decrease in testosterone may result in symptoms such as fatigue, decreased libido, erectile dysfunction, depression, muscle weakness, and others. Unfortunately, these symptoms are not specific to testosterone deficiency (Bhasin et al., 2018). Low testosterone levels are associated with diabetes (Hassanabad & Fatehi, 2018), metabolic syndrome (Mohammed et al., 2018), cardiovascular disease (Corona et al., 2018; Wang et al., 2018), obesity (Molina-Vega et al., 2018), sleep apnea (Viana et al., 2017), and other disorders (Nassar & Leslie, 2023). Additionally, testosterone elevations are associated with serious conditions including tumors, hyperthyroidism, and genetic disorders such as congenital adrenal hyperplasia (Bhasin et al., 2018; Nassar & Leslie, 2023).

Within the serum, testosterone can be either free (i.e., not bound to a specific protein) or protein-bound. Only one to four percent of circulating testosterone is usually found free. SHBG binds testosterone with a high affinity whereas serum albumin, corticosteroid-binding globulin (CBG), and orosomucoid binds testosterone with a much lower affinity. "Bioavailable" testosterone refers to the amount of free testosterone and albumin-bound testosterone as indicated in the figure below (Goldman et al., 2017).



CDC Hormone Standardization (HoSt) Program-Testosterone

Serum testosterone testing can measure either total testosterone (TT) concentration, free testosterone, or bioavailable testosterone. TT can be "measured using radioimmunoassay, immunometric assays, or liquid chromatography-tandem mass spectrometry. Considerable inter-assay and inter-laboratory variability is often found in TT measurements. When 1133 laboratories using 14 different assays measured TT concentrations using the same College of American Pathologists quality control sample from a single hypogonadal man, the measured values ranged from 45 to 365 ng/dL (1.6 to 12.7 nmol/L)" (Bhasin et al., 2018).

The Centers for Disease Control and Prevention (CDC) released their analysis of TT in serum by isotope dilution-liquid chromatography-tandem mass spectrometry (ID-LC-MS/MS) in 2012. As a part of the CDC HoSt Program (CDC Hormone Standardization Program) to certify and calibrate hormone assays, the CDC monitors and validates hormone testing by laboratories and manufacturers. "Calibration is further verified by analyzing serum material with assigned reference values for total testosterone every 6

months and comparing the results obtained against predefined acceptance limit, which is ± 6.4 % from the target value" (CDC, 2012). According to CDC standards, TT using ID/HPLC/MS/MS methodology has a reportable range of 2.5 – 1000 ng/dL or 0.09 – 34.7 nM with a limit of detection of 0.36 ng/dL or 0.012 nM. As for accuracy in terms of "trueness and precision," the CDC reports that total serum testosterone precision (%CV) ranges from 2.2% to 5.5%. The following limitation was noted: "This method was tested for total testosterone analysis in human serum and may not be suitable for other specimens such as plasma, whole blood, urine, and/or saliva. The analytical performance parameters need to be reassessed and verified when other specimen matrices are used" (CDC, 2012). A list of the assays certified by the CDC HoSt/Testosterone program can be found on the CDC's HoSt website (CDC, 2023).

Analytical Validity

A recent study used a liquid chromatography with tandem mass spectrometry (LC-MS-MS) method to assess salivary testosterone, androstenedione, dehydroepiandrosterone, and 17-OH-progesterone. The authors state that the accuracy of this method is "between 83.0 and 106.1% for all analytes" and conclude that this "LC-MS/MS method allowed a sensitive evaluation of androgen salivary levels and represents an optimal technique to explore the relevance of a comprehensive androgen profile as measured in saliva for the study of androgen secretion modulation and activity in physiologic and pathologic states" (Mezzullo et al., 2017). However, another study compared various ELISA-based salivary testosterone assays and noted that "proportional errors between the methods calls [*sic*] for caution" as one of the four methods yielded no results due to malfunction (Andersson et al., 2017). Another study compared salivary testosterone measurements using immunoassays with those measured by tandem mass spectrometry. The authors conclude that the immunoassay-based methods "tended to inflate estimates of lower testosterone concentrations" (Welker et al., 2016).

Recently, van der Veen et al. (2019) developed and validated a LC-MS/MS method to establish reliable reference intervals in five plasma steroid hormones (progesterone, 17-hydroxyprogesterone, androstenedione, testosterone and dihydrotestosterone); these researchers utilized samples from 280 healthy male and female participants over a four-month period. Women taking oral contraceptive pills were found to have lower levels of 17-OH-progesterone and androstenedione; further, it was identified that hormonal biological variation was typically greater in women compared to men (van der Veen et al., 2019). Final conclusions stated that "The gender-specific determination of the reference intervals, together with the observation that the biological variation demonstrated a high degree of variation, allows interpretation of data on individual and group level for improved biochemical characterization of patients in clinical practice" (van der Veen et al., 2019).

Star-Weinstock and Dey (2019) developed an accurate and sensitive method to measure testosterone in hypogonadal adults and children of both genders; this quantification method utilized electrospray ionization (ESI)-LC-MS/MS and achieved a "sensitivity of one ng/dL from 100 µL sample volume." The authors note that two highlights of this novel method are that this sample preparation technique "includes simultaneous protein precipitation and derivatization," and that this TT measurement method was certified by the CDC Hormone Standardization program (Star-Weinstock & Dey, 2019).

Sun et al. (2020) developed and validated an isotope dilution ultra-performance liquid chromatography-tandem mass spectrometry method (ID-UPLC-MS/MS) to measure human serum testosterone. This method offers higher accuracy and lower variability than the traditional immunoassays, especially when measuring low testosterone levels in hypogonadism. To assess accuracy of the method, pure testosterone was added to the serum samples and the actual concentrations after two serial liquid-liquid extractions were measured. The actual concentrations were close to the female and male levels, with a

recovery rate ranging from 94.32 to 108.6%. Sensitivity, specificity, and precision were also measured and met the performance criteria standards established by Clinical and Laboratory Standards Institute (Lynch, 2016) and the Hormone Standardization Program of the Center for Disease Control (Yun et al., 2012). "Moreover, the [ID-UPLC-MS/MS] method exhibited a good consistency between low and high concentrations of testosterone. In addition, the method required a simple sample preparation and a small sample volume, therefore it may be suitable for routine clinical practice" (Sun et al., 2020).

The Centers for Disease Control and Prevention (CDC) reviewed testosterone testing in 2014 in a group of 6746 participants of various age groups and both sexes (Vesper et al., 2014). The positive bias identified by steroid analyte testing indicated that the test was measuring additional compounds (and not only the analyte in question). The authors concluded that "although technologies for steroid hormone measurement have advanced significantly, measurement variability within and across laboratories has not improved accordingly… Within-assay variability for current assays is generally high, especially at low analyte concentrations" (Vesper et al., 2014).

Testosterone and other hormones (AMH, FSH, LH, free androgen index (FAI), prolactin, estradiol) have been used for the clinical diagnosis of polycystic ovary syndrome (PCOS). In a study completed by Khashchenko et al. (2020), 130 girls with PCOS had the accuracy and specificity of hormonal testing assessed and cutoffs for the most significant hormone indicators of PCOS diagnosis in adolescents were identified. The authors found that "Levels of testosterone > 1.15 nmol/L, androstenedione > 11.45 ng/mL, and LH/FSH ratio > 1.23 also showed high sensitivity of 63.2–78.2% and specificity of 84.4–93.7% in PCOS diagnosis in the studied sample of girls" (Khashchenko et al., 2020). The combined use of either four thresholds (AMH, FAI, testosterone, androstenedione, LH/FSH ratio as previously stated) yielded a diagnostic accuracy of 90.2-91.6% in predicting PCOS in adolescents (Khashchenko et al., 2020).

Dalmiglio et al. (2024) evaluated the Vermeulen formula as less expensive and more accessible method of assessing FT than equilibrium dialysis or ultrafiltration. The study included 190 patients, all of whom received FT measurements through both direct immunoluminometric assay and the Vermeulen formula. The authors claim that "the calculated method employing the Vermeulen formula was considered the gold standard." The authors noted that the sensitivity was lower in females, which they claim could be because of a potential proportional bias and the low number of true positive cases. The authors concluded that "the direct method exhibited comparable performance to the calculated method, but caution should be exercised when interpreting results, particularly in females" (Dalmiglio et al., 2024).

Clinical Utility and Validity

Equilibrium dialysis is the gold standard for determining free serum testosterone. Unfortunately, it is technically difficult and has limited availability. Compared to other less accurate methods, it is expensive. It relies on the accuracy and precision of TT determination. In equilibrium dialysis, a semipermeable membrane is used to retain the bound testosterone on one side of the membrane while the free testosterone equilibrates between the two sides. It is dependent on environmental conditions including pH, ionic strength, and temperature; in fact, steroids, such as testosterone, can bind up to 2.5 times higher at 4°C than at 37°C. One study shows that increasing the temperature from 37°C to 41°C increased the free cortisol level by approximately 80% (Goldman et al., 2017).

Immunoassays to measure free and bioavailable testosterone are inaccurate. The Endocrine Society urges the use of medically accepted algorithms that rely on TT, SHBG, and/or albumin to estimate serum free testosterone (Bhasin et al., 2018). Multiple algorithms have been published (Sartorius et al., 2009; Vermeulen et al., 1999; Zakharov et al., 2015). The recent allosteric model proposed by Zakharov and

colleagues models the binding of testosterone as a multi-step, dimeric process. This allosteric model has "close correspondence with those measured using equilibrium dialysis" (Zakharov et al., 2015).

A 2017 international study comprised of multiple cohorts with healthy, non-obese males attempted to "derive standardized, age-specific reference ranges" for circulating testosterone; it was stated that "a substantial proportion of intercohort variation in testosterone levels is due to assay differences" (Travison et al., 2017). Further, the issue in developing standards for circulating testosterone due to variation in body mass and comorbidities was also noted. "Another unresolved issue relates to whether the reference sample should include only the healthy nonobese men or whether it should include the entire population of men 19 to 39 years. Obesity and comorbid conditions affect circulating total testosterone concentrations; therefore, inclusion of obese men with comorbid conditions could distort the reference ranges. Whether the reference ranges generated in nonobese men are appropriate for use in obese men deserves further investigation. Even though men with known diagnoses of conditions or diseases associated with hypogonadism were excluded, it is possible a small percentage of individuals in these cohorts may be hypogonadal" (Travison et al., 2017).

Shukla et al. (2018) organized a cross-sectional study to measure the relationship between prostatic specific antigen (PSA) and serum testosterone levels in both healthy men and men with partial androgen deficiency (PADAM); a total of 255 men participated in this study. "Mean total testosterone and serum PSA was 9.35 ± 1.33 nmol/L, 1.96 ± 0.76 ng/mL in males with PADAM and 15.30 ± 1.95 nmol/L, 1.85 ± 0.73 ng/mL respectively in males without PADAM. No significant relationship was observed between serum PSA and serum testosterone levels among healthy males irrespective of PADAM" (Shukla et al., 2018). Results from this study suggest that PSA values do not need to be adjusted "for biopsy decisions according to testosterone levels" (Shukla et al., 2018).

In a retrospective cohort study, eighty-five severely hypogonadal men were observed for changes in serum prostate specific antigen (PSA) concentrations during testosterone treatment for 18 months. The Endocrine Society clinical guidelines recommend measuring PSA in hypogonadal men over the age of 50 at three months and twelve months after starting testosterone therapy and urologic referral if serum PSA > 1.4 ng/mL above baseline or to an absolute value > four ng/mL (Bhasin et al., 2018). Studies have been performed in men with mild to moderate hypogonadism which reported smaller increases in serum PSA concentrations during testosterone treatment; however, no studies have reported serum PSA changes in response to testosterone treatment of severely hypogonadal men. In this study, testosterone treatment "increased the median serum testosterone concentration from 36 ng/dL at baseline to 395 ng/dL at 6-18 months. This treatment resulted in a median increment in PSA above baseline of 0.70 ng/mL at 6-18 months…31% of men had increases in PSA >1.4 ng/mL; and 13% of men reached absolute PSA concentrations > four ng/mL" (Sachdev et al., 2020). The authors suggest that "testosterone treatment of severely hypogonadal men often increases PSA above the commonly accepted thresholds for urologic referral [and] that future clinical guidelines for the expected PSA response to testosterone replacement reflect the degree of hypogonadism" (Sachdev et al., 2020).

A total of nine years of registry data, comprised of 650 patients with hypogonadism, was analyzed to determine the impact of long-term intramuscular testosterone treatment (1000 mg every 10-12 weeks) (Zitzmann et al., 2019). Serum testosterone concentrations were found to increase "from 5.7±2.3 nmol/L to 19.4±2.8 nmol/L in men with classical hypogonadism and from 7.8±2.4 nmol/L to 19.2±3.1 nmol/L in men with functional hypogonadism"; final conclusions suggest that patients with the functional form of hypogonadism may benefit the most from testosterone treatment as "men with functional hypogonadism were more likely to lose ten percent weight and five percent of waist circumference (WC)

than men with classical hypogonadism" (Zitzmann et al., 2019). Men with functional hypogonadism were also more likely to be obese at the start of the study.

Cauley et al. (2021) performed a study to examine the effect of testosterone treatment on TBS. "Two hundred and eleven men were enrolled in Bone Trial of the Testosterone Trials. Of these, 197 men had two repeat TBS and vBMD measurements; 105 men were allocated to receive testosterone, and 92 men to placebo for one year. TBS, a BMD, and vBMD were assessed at baseline and month 12." The results of this study report that there was no difference in the percent change in TBS by randomized group. They saw a 1.6% (95% confidence intervals (CI) 0.2–3.9) change in the testosterone group and a 1.4% (95% CI−0.2, 3.1) change in the placebo group. In contrast, they saw a six percent increase in vBMD (95% CI4.5–7.5) in the testosterone group as compared to only a 0.4% vBMD change (95% CI−1.65–0.88) in the placebo groups (Cauley et al., 2021). As a result, the authors concluded that TBS was not clinically useful in monitoring the one year effect of testosterone treatment on the bone structure in older hypogonadal men (Cauley et al., 2021).

Stern and Casto (2024) studied the differences in salivary testosterone levels across the menstrual cycle. A total of 339 people with a menstrual cycle and confirmed ovulation were included. Salivary testosterone was measured with LC-MS/MS four times across the mid-cycle ovulatory window during the luteal phase. "Within-subject analysis revealed a significant but small pattern of a mid-cycle peak and a luteal decrease at the aggregate level." The authors note that at the individual level, there was "substantial variability between the direction and magnitude of the testosterone-cycle pattern." The authors conclude that "salivary testosterone levels show a small trend towards a mid-cycle peak compared to the earlier follicular phase and the later luteal phase of the menstrual cycle when looking at the aggregate across all participants," but overall, "menstrual patterns of testosterone appear subtle and not systematic across individuals" (Stern & Casto, 2024).

Maimoun et al. (2011) studied the diagnosis process of 5-alpha reductase deficiency. The study included 55 patients with srd5A2 gene mutations. The authors found a "wide spectrum" of phenotypes, including clitoromegaly in 49.1% of participants, microphallus with various degrees of hypospadias in 32.7% of participants, female external genitalia in 7.3% of participants and isolated micropenis in 3.6% of participants. Overall, "over 72% of patients were considered for 5α-reductase deficiency diagnosis when the testosterone/dihydrotestosterone cutoff was 10" (Maimoun et al., 2011).

Imperato-McGinley et al. (1986) studied the clinical criteria used to diagnosis three infants with 5-alpha reductase deficiency. Initially, "basal plasma testosterone to dihydrotestosterone ratios were significantly elevated in two of the three affected infants, and increased markedly in all three infants after administration of hCG." The authors note that urinary etiocholanolone to androsterone ratios could not be accurately measured in infants, so the diagnosis was confirmed by using gas chromatography/mass spectrometry to measure urinary tetrahydrocortisol (THF) to 5 alpha-tetrahydrocortisol (5 alpha-THF) ratios. "The affected infants had THF/5 alpha-THF ratios comparable to ratios in adult carrier males and significantly lower than ratios in adult homozygotes." The authors concluded that 5 alpha reductase is detectable at infancy (Imperato-McGinley et al., 1986).

Guidelines and Recommendations

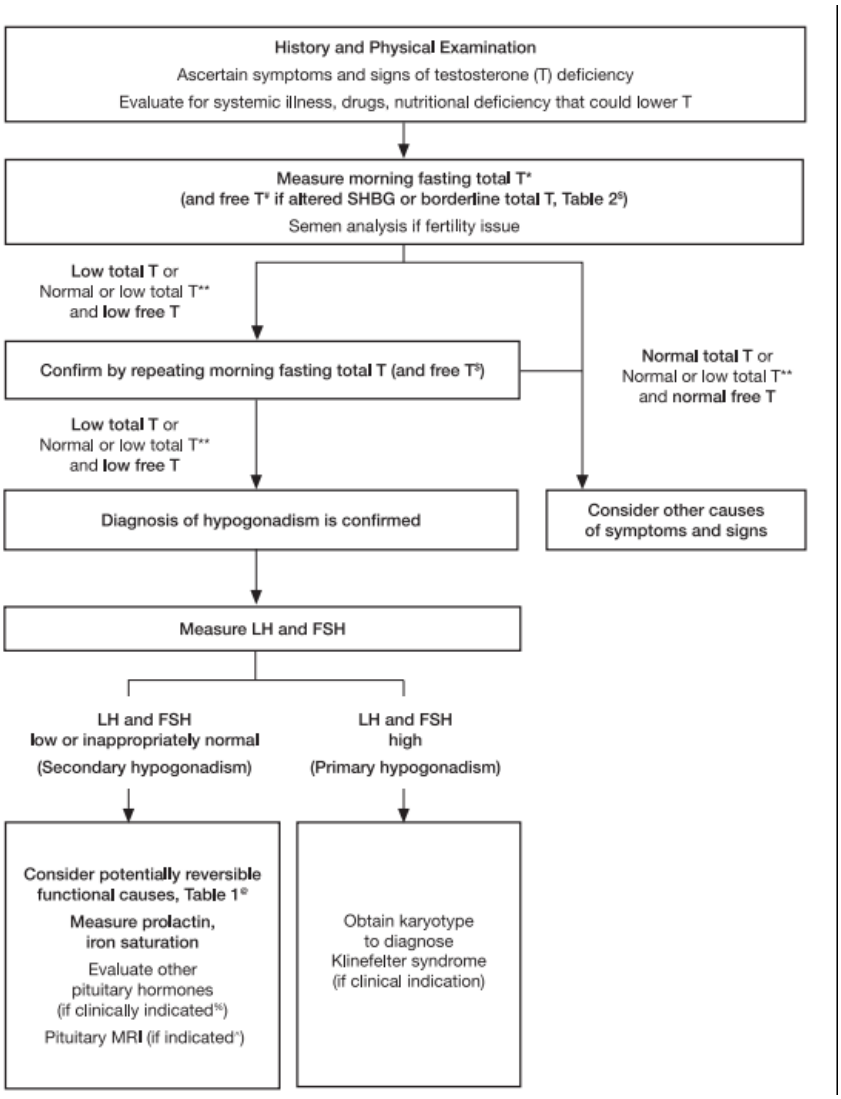
The Endocrine Society (ES)

Androgen Deficiency Testosterone Therapy

The ES, in updated 2018 guidelines concerning testosterone therapy and hypogonadism in males, summarized their recommendations with respect to testosterone testing with the following:

- Recommendation 1.1: "We recommend diagnosing hypogonadism in men with symptoms and signs of testosterone deficiency and unequivocally and consistently low serum total testosterone and/or free testosterone concentrations (when indicated)." (Level 1+++)
- Recommendation 1.2: "We recommend against routine screening of men in the general population for hypogonadism." (Level 1++)
- Recommendation 1.3: "In men who have hypogonadism, we recommend distinguishing between primary (testicular) and secondary (pituitary–hypothalamic) hypogonadism by measuring serum luteinizing hormone and follicle-stimulating hormone concentrations." (Level 1+++)
- Recommendation 1.4: "In men with hypogonadism, we suggest further evaluation to identify the etiology of hypothalamic, pituitary, and/or testicular dysfunction." (Level 2++)
- Recommendation 3.1: "In hypogonadal men who have started testosterone therapy, we recommend evaluating the patient after treatment initiation to assess whether the patient has responded to treatment, is suffering any adverse effects, and is complying with the treatment regimen." (Ungraded Good Practice Statement)

Within the explanations and technical comments of the recommendations, the ES specifically states, "Clinicians should not use direct analog-based free testosterone immunoassays, as they are inaccurate." Moreover, recommendations state that serum total testosterone testing is preferred and should be performed on two separate days after fasting since testosterone concentrations can vary due to many circumstances. For men who initially test low TT, who test near the lower limit, or who have conditions associated with altering SHBG, then free testosterone can be measured either by using an accepted algorithm based on the TT, SHBG, and albumin concentrations or by direct equilibrium dialysis methods rather than the use of immunoassays. As for bioavailable testosterone testing, the ES states, "Measuring bioavailable [testosterone] concentrations using ammonium sulfate precipitation is technically challenging. Furthermore, there are no detailed studies (similar to those described previously that relate FT [free testosterone] concentrations to manifestations of [testosterone] deficiency) that use bioavailable [testosterone] concentrations." Men beginning hormone replacement therapy should have their serum testosterone and hematocrit levels measured initially to establish a baseline and then, depending on the therapy, have the levels measured again three to six months later. While on testosterone therapy, the TT and hematocrit levels should be checked annually thereafter. Concerning secondary hypogonadism, serum prolactin and either serum ferritin or iron saturation measurements are recommended to check for the possibility of reversibility of the condition. The testing algorithm also recommends testing serum LH and FSH to differentiate primary and secondary hypogonadism. The algorithm for testing for hypogonadism is shown in the figure below (Bhasin et al., 2018):



Hypothalamic Amenorrhea: An Endocrine Society Clinical Practice Guideline

Testosterone testing in addition to other endocrine laboratory tests is recommended as part of an initial endocrine assessment for women with clinical hyperandrogenism in the evaluation of suspected functional hypothalamic amenorrhea (FHA). FHA is a condition of anovulation, in which the ovary fails to release an egg during the menstrual cycle and has been correlated with stress, weight loss, and excessive exercise (Gordon et al., 2017).

Polycystic Ovary Syndrome (PCOS)

Relative to the diagnosis of PCOS, the ES identifies three criteria that may be evaluated: androgen excess, ovulatory dysfunction, and polycystic ovaries. Two of the three criteria are sufficient for diagnosis, and if both clinical criteria are met, they do not recommend testing for androgen excess. Androgen excess is characterized by elevated serum androgen levels such as elevated total, bioavailable, or free serum testosterone levels. Considering that serum testosterone levels are variable and there is poor standardization of assays, the Task Force recommends familiarity with local assays and does not define an absolute level that is diagnostic of PCOS or other causes of hyperandrogenism (Legro et al.,

2013).

Endocrine Treatment of Gender-Dysphoric/Gender-Incongruent Persons

The ES published guidelines suggesting that testosterone level monitoring is suggested at baseline and every 6-12 months during suppression of puberty treatment protocol in gender-dysphoric/gender-incongruent persons. The laboratory monitoring of testosterone levels is also suggested at baseline and every 6-12 months during induction of puberty protocol. Measurement of serum testosterone levels is suggested every three months until levels are in the normal physiologic male range during the monitoring of transgender males on gender-affirming hormone therapy. Testosterone testing is also needed midway between injections for monitoring of testosterone enanthate/cypionate injections, alternatively peak and trough levels could be measured to ensure levels remain in the normal male range. For parenteral testosterone undecanoate, testosterone should be measured just before the following injection. For transdermal testosterone, the testosterone level can be measured no sooner than after one week of daily application (at least 2h after application). For monitoring transgender females on gender-affirming hormone therapy, measurement of serum testosterone is indicated every three months (Hembree et al., 2017).

The American College of Obstetricians and Gynecologists

In 2018, the ACOG released guidelines on the clinical management of polycystic ovary syndrome (PCOS). In its suggested evaluation of patients with PCOS, the ACOG recommends having a physical, laboratory testing, and an ultrasound examination to confirm the polycystic ovaries. With regards to hormone testing, it includes "documentation of biochemical hyperandrogenemia" by "total testosterone and sex-hormone binding globulin or bioavailable and free testosterone," but notes to conduct testing that would exclude other causes of hyperandrogenism, such as thyroid dysfunction and hyperprolactinemia. ACOG includes TSH, prolactin, and 17-hydroxyprogesterone as hormones to measure to exclude other causes. The ACOG (2018b) also acknowledges that "there is no standardized testosterone assay in the United States and the sensitivity and reliability in the female ranges are often poor."

Regarding Müllerian Agenesis, ACOG writes that the initial evaluation of a patient without a uterus "may include the following laboratory tests: testosterone level, FSH level, and karyotype" (ACOG, 2018a).

In 2019, ACOG released a guideline regarding the "screening and management of the hyperandrogenic adolescent." In it, they state that the diagnosis of hyperandrogenism can be based on clinical symptoms or measurement of serum androgens. However, they recommend against monitoring serum androgens. This guideline was reaffirmed in 2024.

The ACOG recommends identifying clinical symptoms of androgen excess during the initial evaluation. In the proposed algorithm for evaluation, ACOG recommends two separate batteries of hormone tests depending on type of menses. For regular menses, ACOG lists free and TT, DHEAS (dehydroepianandrosterone sulphate), and 17OHP (17-α-hydroxyprogesterone) as hormones that may be tested. For irregular menses, ACOG lists prolactin, LH, FSH, TSH, and the three previously mentioned hormones. ACOG also notes that PCOS may be one of the diagnoses if both androgen excess and irregular menses are identified (ACOG, 2019). This guideline was reaffirmed in 2024.

American Urological Association (AUA)

The AUA published guidelines concerning the evaluation and management of testosterone deficiency in 2018. Five recommendations are given concerning the diagnosis of testosterone deficiency:

1. "Clinicians should use a total testosterone level below 300 ng/dL as a reasonable cut-off in support of the diagnosis of low testosterone. (Moderate Recommendation; Evidence Level: Grade B)
2. The diagnosis of low testosterone should be made only after two total testosterone measurements are taken on separate occasions with both conducted in an early morning fashion. (Strong Recommendation; Evidence Level: Grade A)
3. The clinical diagnosis of testosterone deficiency is only made when patients have low total testosterone levels combined with symptoms and/or signs. (Moderate Recommendation; Evidence Level: Grade B)
4. Clinicians should consider measuring total testosterone in patients with a history of unexplained anemia, bone density loss, diabetes, exposure to chemotherapy, exposure to testicular radiation, HIV/AIDS, chronic narcotic use, male infertility, pituitary dysfunction, and chronic corticosteroid use even in the absence of symptoms or signs associated with testosterone deficiency. (Moderate Recommendation; Evidence Level: Grade B)
5. The use of validated questionnaires is not currently recommended to either define which patients are candidates for testosterone therapy or to monitor symptom response in patients on testosterone therapy. (Conditional Recommendation; Evidence Level: Grade C)"

Other recommendations by the AUA concerning adjunctive testing in males include the following:

6. "In patients with low testosterone, clinicians should measure serum luteinizing hormone levels (Strong Recommendation; Evidence Level: Grade A)
7. Serum prolactin levels should be measured in patients with low testosterone levels combined with low or low/normal luteinizing hormone levels (Strong Recommendation; Evidence Level: Grade A)
8. Patients with persistently high prolactin levels of unknown etiology should undergo evaluation for endocrine disorders (Strong Recommendation; Evidence Level: Grade A)
9. Serum estradiol should be measured in testosterone deficient patients who present with breast symptoms or gynecomastia prior to the commencement of testosterone therapy. (Expert Opinion)
10. Men with testosterone deficiency who are interested in fertility should have a reproductive health evaluation performed prior to treatment. (Moderate Recommendation; Evidence Level: Grade B)
11. Prior to offering testosterone therapy, clinicians should measure hemoglobin and hematocrit and inform patients regarding the increased risk of polycythemia. (Strong Recommendation; Evidence Level: Grade A)
12. PSA should be measured in men over 40 years of age prior to commencement of testosterone therapy to exclude a prostate cancer diagnosis. (Clinical Principle)" (Mulhall et al., 2014).

American Academy of Pediatrics (AAP) —Choosing Wisely Initiative

As a part of the *Five Things Physicians and Patients Should Question* series of the Choosing Wisely initiative of the American Board of Internal Medicine (ABIM) foundation, the AAP states the following: "Avoid ordering LH and FSH and either estradiol or testosterone for children with pubic hair and/or body odor but no other signs of puberty." Further, "premature adrenarche is usually the diagnosis and does not involve activation of the pituitary- gonadal axis but is due to an early increase in adrenal androgens. DHEA-S levels are elevated for age but do not alter the management of this common and generally benign condition" (AAP, 2017).

European Academy of Andrology (EAA)

The EAA published guidelines concerning management of bone health in males and testing in *Andrology*, a journal jointly published by the EAA and the American Society of Andrology. Recommendations include the following:

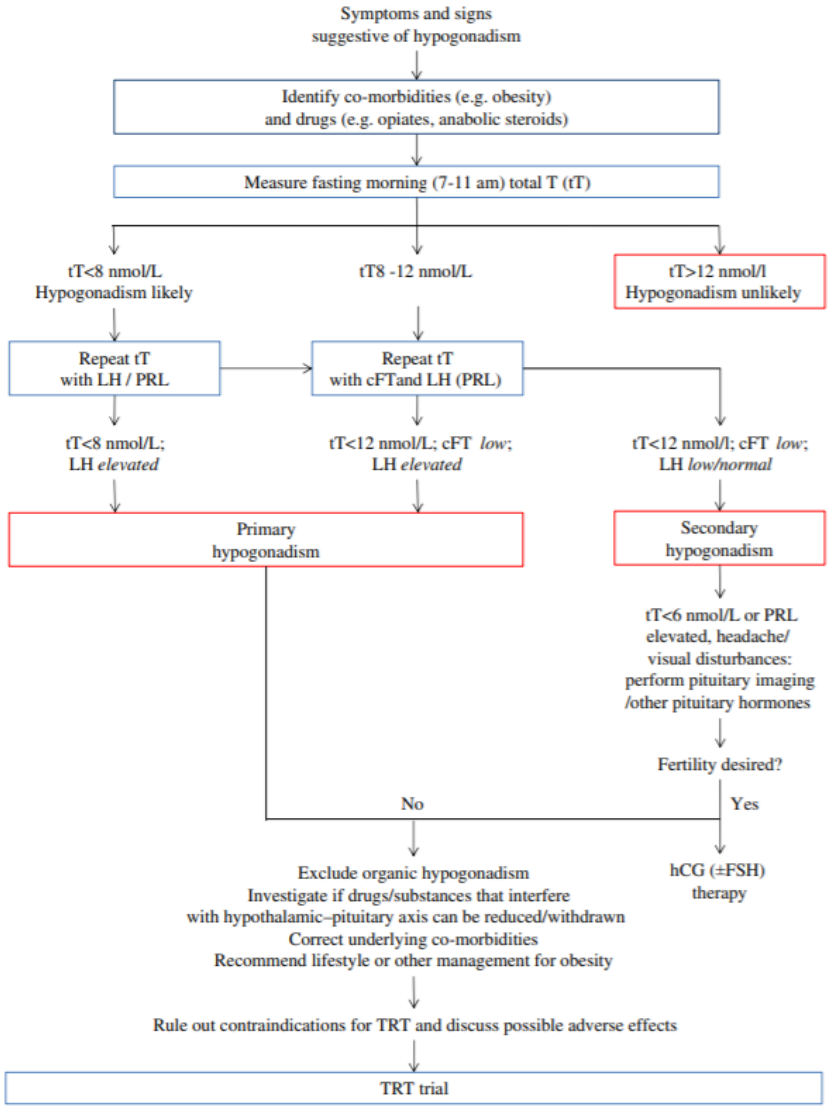
- "We recommend having serum total testosterone measured twice on a morning blood sample." (Level 1+++)
- "We recommend measuring again total testosterone and SHBG if only a single measurement documenting low testosterone is available. LH and prolactin are useful to better characterize hypogonadism." (Level 1+++)
- "We do not recommend routine measurement of serum estradiol." (Level 1++)
- "We suggest measuring estradiol only when a validated mass spectrometry-based method is available and in rare cases in which severe estrogen deficiency is suspected." (Level 2++)

Within the evidence and rationale behind the recommendations, the EAA goes on to state, "We suggest using calculated free testosterone when needed, based on the measurement of total serum testosterone, SHBG, and albumin... It can easily be obtained using online available calculators (see Appendix 2 [of (Rochira et al., 2018)] for Web links). Commercially available kits for direct measurement of free testosterone should not be used due to their poor accuracy and reliability" (Rochira et al., 2018). Concerning other hormones, the EEA states, "all patients with documented low serum testosterone consulting with hypogonadal symptoms should receive a biochemical evaluation of their gonadal status, with measurement of serum total testosterone, SHBG, luteinizing hormone (LH), follicle-stimulating hormone (FSH), and prolactin" (Rochira et al., 2018).

The EAA also published clinical practice guidelines regarding gynecomastia evaluation and management. The EAA recommended testing several hormones for gynecomastia including "testosterone (T), estradiol (E2), sex hormone-binding globulin (SHBG), luteinizing hormone (LH), follicular stimulating hormone (FSH), thyroid stimulating hormone (TSH), prolactin, human chorionic gonadotropin (hCG), alpha-fetal protein (AFP), liver and renal function tests" (Kanakis et al., 2019).

The EAA recently published clinical practice guidelines on investigation, treatment, and monitoring of functional hypogonadism in males to provide certain recommendations:

1. "We recommend against universal screening for hypogonadism in middle-aged or older men, by structured interviews or questionnaires and/or random total T measurements.
2. We recommend that the clinical diagnosis of functional hypogonadism should be confirmed by measurement of serum total T with a well validated assay on fasting morning (before 11 am) blood samples obtained on two different days.
3. Functional hypogonadism should be diagnosed only after exclusion of organic causes of hypogonadism. In addition, to morning total T, luteinizing hormone (LH) should be measured in all patients with suspected functional hypogonadism to differentiate between the primary and secondary causes.
4. We recommend either measuring or calculating free T (fT), in addition to total T, in patients with conditions that alter sex hormone-binding globulin (SHBG) and when total T concentrations are in the borderline range (~8-12 nmol/L) if the clinical suspicion of hypogonadism is strong" (Corona et al., 2020).



Above, is a proposed flow chart to diagnose and manage functional hypogonadism (Corona et al., 2020).

Canadian Urological Association (CUA) and Canadian Society of Endocrinology and Metabolism (CSAM)

The CUA and CSAM endorsed joint guidelines published in the *CMAJ* in 2015. The following recommendations were given concerning hormone testing in males for testosterone deficiency syndrome (Morales et al., 2015):

- "We recommend a thorough history and physical examination, instead of the exclusive reliance on standard questionnaires, to identify patients requiring biochemical testing (strong recommendation; moderate-quality evidence)."
- "The initial biochemical test should be total testosterone level measured in serum samples taken in the morning; determinations of bioavailable testosterone or free testosterone should be

restricted to patients with equivocally low total testosterone levels (strong recommendation; high-quality evidence)."

- "We recommend that sample collection for testosterone measurement occur between seven am and eleven am, or within three hours after waking in the case of shift workers (strong recommendation; moderate-quality evidence)."
- "Testosterone levels should be measured with the use of testosterone assays traceable to internationally recognized standardized reference material; commercial assays should be certified by the testosterone standardization program of the US Centers for Disease Control and Prevention (strong recommendation; high-quality evidence)."
- "Measurement of sex hormone-binding globulin with calculated free or bioavailable testosterone should be restricted to men with symptoms of testosterone deficiency and equivocally low testosterone levels (strong recommendation; moderate-quality evidence)."
- "We recommend investigation for secondary or reversible causes of hypogonadism in all men with testosterone deficiency syndrome (strong recommendation; moderate-quality evidence)."
- "We recommend investigation for testosterone deficiency syndrome and treatment with testosterone in men with anemia or sarcopenia of undetermined origin (strong recommendation; moderate-quality evidence)."
- "We recommend assessment of response and adverse effects at three and six months after onset of therapy (strong recommendation; high-quality evidence)."
- "Testosterone levels should be assessed at three and six months after onset of therapy and then annually thereafter if stable (weak recommendation; low-quality evidence)."

European Association of Urology (EAU)

In the 2014 EAU guidelines concerning the treatment of castration-resistant prostate cancer, the EAU states, "Follow-up after ADT should include analysis of PSA and testosterone levels, and screening for cardiovascular disease and metabolic syndrome" (Heidenreich et al., 2014).

The EAU released guidelines on sexual and reproductive health and expressed the following recommendations for diagnosis of hypogonadism:

1. "Check for concomitant diseases, drugs and substances that can interfere with testosterone production/action.
2. Measure total testosterone in the morning (between 07.00 and 11.00 hours) and in the fasting state, with a reliable laboratory assay.
3. Repeat total testosterone on at least two separate occasions when < 12 nmol/L and before starting testosterone therapy.
4. Use 12 nmol/L total testosterone (3.5 ng/mL) as a reliable threshold to diagnose late onset hypogonadism (LOH).
5. Measure sex hormone-binding globulin and free-testosterone calculation when indicated.
6. Analyse luteinising hormone and follicle-stimulating hormone serum levels to differentiate between the different types of hypogonadism.
7. Measure prolactin (PRL) levels if evidence of low sexual desire (or other suggestive signs/ symptoms) and secondary hypogonadism is present.
8. Perform pituitary magnetic resonance imaging (MRI) in secondary hypogonadism, with elevated PRL or symptoms specific of a pituitary mass and/or presence of other anterior pituitary hormone deficiency.
9. Perform pituitary MRI in secondary severe hypogonadism (total testosterone < 6 nmol/L)..
10. Screen for late onset hypogonadism (LOH) only in symptomatic men.

11. Do not use structured interviews and self-reported questionnaires for systematic screening for LOH as they have a low specificity."

The EAU recommends that the standard and most accurate method for testosterone serum testing is liquid chromatography-tandem mass spectrometry (LC-MS/MS). Standardized automated platform immuno-assays are reliable techniques to measure testosterone; however, only LC-MS/MS can provide an accurate measurement of FT (fT) levels. When diagnosing late-onset hypogonadism, the EAU recommends measuring fasting and morning (7-11am) TT, noting to "(consider PRL measurement if low desire or other suggestive symptoms are present," "consider SHBG and free-T calculations when indicated," consider LH when T deficiency pathophysiology must be investigated." There is uncertainty as to what threshold of fT level indicates hypogonadism, but some data indicates that fT levels below 225 pmol/L is associated with hypogonadism (Salonia et al., 2024).

National Comprehensive Cancer Network

Within the algorithm concerning the systemic therapy for castration-naïve disease, the NCCN says to "document castrate level of testosterone if clinically indicated" when assessing progression along with the physical exam and PSA every three to six months. The NCCN also states to "continue ADT [androgen deprivation therapy] to maintain castrate serum levels of testosterone (<50 ng/dL)." Additional recommendations state, "close monitoring of PSA and testosterone levels and possibly imaging is required when using intermittent ADT, especially during off-treatment periods, and patients may need to switch to continuous ADT upon signs of disease progression" (NCCN, 2024b).

The NCCN also published some guidance regarding assessment of hormones for neuroendocrine and adrenal tumors. For pituitary tumors, they list serum prolactin and LH/FSH; for "suspected or confirmed adrenocortical carcinoma", they list "screen for hypercortisolemia (± Cushing syndrome) and primary aldosteronism" and "adrenal androgens (DHEAS, androstenedione, testosterone, 17-hydroxyprogesterone)"; for hypercortisolemic Cushing's Syndrome, they list "screen for hypercortisolemia (± Cushing syndrome) with 1 of the following tests: 1 mg overnight dexamethasone suppression test, 2–3 midnight salivary cortisols, [or] 24-hour urinary free cortisol" and "plasma ACTH [adrenocorticotropic hormone] in AM if confirmed hypercortisolemia (± Cushing syndrome)" (NCCN, 2024a).

International Late Effects of Childhood Cancer Guideline Harmonization Group (IGHG) & PanCareSurFup (PCSF) Consortium

Within the guidelines and recommendations issued in 2017 by the IGHG and the PCSF Consortium for patients with possible impaired spermatogenesis, it is recommend that "Clinical measurement of testicular volume and of follicle-stimulating hormone and inhibin B might be reasonable for the identification of impaired spermatogenesis in survivors treated with potentially gonadotoxic chemotherapy or radiotherapy potentially exposing the testes in whom semen analysis has been declined or is not possible and who desire assessment about possible future fertility. Be aware of the diagnostic limitations of these tests that may result in false positives or false negatives (level B evidence)." With respect to patients with possible testosterone deficiency, "Measurement of testosterone concentration in an early morning blood sample at clinically appropriate intervals is reasonable in post pubertal survivors treated with radiotherapy potentially exposing the testes to 12 Gy or more or with TBI (expert opinion). In the presence of clinical signs of hypogonadism, or of previous low-normal or borderline testosterone concentrations, or if it is not possible to obtain an early morning blood sample,

it is reasonable to measure luteinising hormone concentration in addition to testosterone (expert opinion)" (Skinner et al., 2017).

American Society of Reproductive Medicine (ASRM)

The ASRM, in collaboration with the Society for Male Reproduction and Urology, released a committee opinion on the diagnostic evaluation of sexual dysfunction in the male partner in the setting of infertility. The publication recommends the following for the detection of erectile dysfunction: "A physical examination should include blood pressure and the calculation of body mass index, as well as an assessment for signs of testosterone deficiency. Morning serum testosterone should be assayed, as should glucose and hemoglobin A1c levels, as well as lipid profile measurements, as indicated" (ASRM, 2023).

Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
82040	Albumin; serum, plasma or whole blood
82642	Dihydrotestosterone (DHT)
82670	Estradiol; total
82681	Estradiol; free, direct measurement (eg, equilibrium dialysis)
84270	Sex hormone binding globulin (SHBG)
84402	Testosterone; free
84403	Testosterone; total
84410	Testosterone; bioavailable, direct measurement (eg, differential precipitation)

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

Evidence-based Scientific References

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Revision History

Revision Date	Summary of Changes
12/04/2024	Reviewed and Updated: Updated the background, guidelines and recommendations, and evidence-based scientific references. Literature review did not necessitate any modifications to coverage criteria.

Thyroid Disease Testing

Policy Number: AHS – G2045 – Thyroid Disease Testing	Prior Policy Name and Number, as applicable:
Initial Presentation Date: 03/19/2015 Effective Date: February 1, 2025	

Policy Description

Thyroid hormones are necessary for both prenatal and postnatal development, as well as metabolic activity in adults (Brent, 2024).

Thyroid disease includes conditions which cause hypothyroidism, hyperthyroidism, goiter, thyroiditis (which can present as either hypo- or hyperthyroidism), and thyroid tumors (Rugge et al., 2015).

Thyroid function tests are used in a variety of clinical settings to assess thyroid function, monitor treatment, and screen asymptomatic populations for subclinical or otherwise undiagnosed thyroid dysfunction (Ross, 2023b).

Terms such as male and female are used when necessary to refer to sex assigned at birth.

Related Policies

Policy Number	Policy Title
AHS-G2035	Prenatal Screening (Nongenetic)
AHS-G2042	Pediatric Preventive Screening
AHS-M2108	Molecular Markers in Fine Needle Aspirates of the Thyroid

Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) Thyroid function testing **MEETS COVERAGE CRITERIA** in the following situations:
    a) For individuals with signs and symptoms consistent with hypothyroidism (see Note 1):
        i) Thyroid stimulating hormone (TSH) testing to confirm or rule out primary hypothyroidism.
        ii) Free T4 (fT4) testing as a follow up to abnormal TSH finding.
        iii) TSH and fT4 testing in cases of suspected secondary hypothyroidism.
        iv) For individuals being treated for primary hypothyroidism, monitoring with TSH and fT4 testing every 6 weeks upon dosage change and annually in stable individuals.
        v) For individuals being treated for secondary hypothyroidism, monitoring with fT4 testing every 6 weeks upon dosage change and annually in stable individuals.
    b) For individuals with signs and symptoms consistent with hyperthyroidism (see Note 2):
        i) TSH testing to confirm or rule out overt hyperthyroidism.
        ii) fT4 testing as a follow up to abnormal TSH findings.
        iii) Total T3 (TT3) or free T3 (fT3) testing to confirm a diagnosis of hyperthyroidism.
        iv) fT4 testing to distinguish between overt and subclinical hyperthyroidism.
        v) Monitoring individuals after treatment for hyperthyroidism:
            (a) In patients being treated for hyperthyroidism, repeat testing of TSH and fT4 should occur every 8 weeks.
            (b) Annual monitoring after first year even if asymptomatic for risk of relapse or late-onset hypothyroidism.
    c) For asymptomatic individuals who have been prescribed drugs that can interfere with thyroid function and thus who are at an increased risk for thyroid disease, TSH testing at the following intervals:
        i) Annually.
        ii) When dosage or medication changes.
        iii) If symptoms consistent with thyroid dysfunction develop.
    d) TSH testing for individuals capable of becoming pregnant who:
        i) Are undergoing evaluation for infertility.
        ii) Have experienced two or more pregnancy losses.
    e) One-time TSH screening:
        i) For asymptomatic individuals at high risk for thyroid disease due to:
            (a) Personal or family history of thyroid dysfunction.

(b) Personal or family history of type 1 diabetes or other autoimmune disease.

ii) For individuals with disease or neoplasm of the thyroid or other endocrine glands.

iii) For individuals with chronic or acute urticaria.

iv) For pediatric individuals diagnosed with short stature.

v) For pediatric individuals with a clinical finding of failure-to-thrive.

f) TSH testing once every 3 months, with reflex fT4 and fT3 when TSH is abnormal, for individuals undergoing immune reconstitution therapy (IRT):

i) Individuals with active relapsing remitting multiple sclerosis (MS) undergoing therapy with alemtuzumab (Lemtrada).

ii) Individuals with HIV undergoing highly active antiretroviral therapy (HAART).

iii) Individuals following allogeneic bone marrow transplantation (BMT) or hematopoietic stem cell transplantation (HSCT).

g) For individuals with hypothalamic-pituitary disease, monitoring of TSH and fT4:

i) Biannually for individuals less than 18 years of age.

ii) Annually for individuals 18 years of age or older.

h) Annual screening of TSH and fT4 for individuals diagnosed with primary mitochondrial disease.

a)

2) For individuals who are pregnant or who are postpartum and who have symptoms of thyroid dysfunction (see Note 1 and Note 2), TSH and fT4 testing (once every 4 weeks) **MEETS COVERAGE CRITERIA** (see Note 3).

3) For individuals who are pregnant or who are postpartum and who have been diagnosed with hyperthyroidism, total T4 (TT4), antithyroglobulin antibody (Tg-Ab), thyrotropin receptor antibodies (TRAb), and anti-thyroid peroxidase antibody (TPOAb) **MEETS COVERAGE CRITERIA** (see Note 3).

4) For individuals with hypothyroidism or hyperthyroidism, testing for thyroid antibodies (once every three years) **MEETS COVERAGE CRITERIA**.

5) For individuals with thyroid cancer, testing for serum thyroglobulin and/or Tg-Ab levels for the detection of tumor recurrence, post-surgical evaluation, surveillance, and maintenance for differentiated thyroid carcinomas **MEETS COVERAGE CRITERIA**.

6) For the evaluation of the cause of hyperthyroidism or hypothyroidism, testing for thyrotropin-releasing hormone (TRH) or thyroxine-binding globulin (TBG) **DOES NOT MEET COVERAGE CRITERIA**.

7) For all other situations not mentioned above, testing of reverse T3, T3 uptake, and TT4 **DOES NOT MEET COVERAGE CRITERIA**.

8) For the assessment of hypothyroidism, measurement of TT3 and/or fT3 **DOES NOT MEET COVERAGE CRITERIA**.

9) To assess levothyroxine dose in hypothyroid patients, measurement of total or fT3 level **DOES NOT MEET COVERAGE CRITERIA**.

10) For asymptomatic nonpregnant individuals, testing for thyroid dysfunction during a general exam without abnormal findings **DOES NOT MEET COVERAGE CRITERIA**.

NOTES:

Note 1: Signs and symptoms of hypothyroidism include:

- Fatigue
- Increased sensitivity to cold
- Constipation
- Dry skin
- Unexplained weight gain
- Puffy face
- Hoarseness
- Muscle weakness
- Elevated blood cholesterol level
- Muscle aches, tenderness, and stiffness
- Pain, stiffness or swelling in your joints
- Heavier than normal or irregular menstrual periods
- Thinning hair
- Slowed heart rate
- Depression
- Impaired memory

Note 2: Hyperthyroidism can mimic other health problems, which may make it difficult for doctors to diagnose. It can also cause a wide variety of signs and symptoms, including:

- Sudden weight loss, even when your appetite and the amount and type of food you eat remain the same or even increase
- Rapid heartbeat (tachycardia) — commonly more than 100 beats a minute — irregular heartbeat (arrhythmia) or pounding of your heart (palpitations)
- Increased appetite
- Nervousness, anxiety, and irritability
- Tremor — usually a fine trembling in your hands and fingers
- Sweating
- Changes in menstrual patterns
- Increased sensitivity to heat
- Changes in bowel patterns, especially more frequent bowel movements
- An enlarged thyroid gland (goiter), which may appear as a swelling at the base of your neck
- Fatigue, muscle weakness
- Difficulty sleeping
- Skin thinning
- Fine, brittle hair

Note 3: Due to significant changes in thyroid physiology during pregnancy, measurement of hormone levels should only be performed at labs that have trimester specific normal ranges for their assay(s). While fT4 is the preferred test, TT4 may be useful if the TSH and fT4 results are discordant or when trimester specific normal ranges for fT4 are unavailable.

Table of Terminology

Term	Definition
AAAAI	Academy of Allergy, Asthma & Immunology
AACE	American Association of Clinical Endocrinologists
AAFP	American Academy of Family Physicians
AAP	American Academy of Pediatrics
AAAAI	American College of Allergy, Asthma & Immunology
ACOG	American College of Obstetricians and Gynecologists
AITD	Autoimmune thyroid disease
AJGP	Australian Journal of General Practice
ALPS	Autoimmune lymphoproliferative syndrome
Anti-TPO	Anti-thyroid peroxidase antibodies
ASCP	American Society for Clinical Pathology
ATA	American Thyroid Association
ATD	Antithyroid drug treatment
ATDs	Antithyroid drugs
BMI	Body mass index
BMT	Bone marrow transplantation
CeH	Central hypothyroidism
CFPC	College of Family Physicians of Canada
CLIA '88	Clinical Laboratory Improvement Amendments of 1988
CMS	Centers for Medicare and Medicaid Services
CSEM	Canadian Society of Endocrinology and Metabolism
CTFPHC	Canadian Task Force on Preventive Health Care
DNA	Deoxyribonucleic acid
ELISA	Enzyme-linked immunosorbent assay
ES	Endocrine Society
ETA	European Thyroid Association
fT3/FT3	Free triiodothyronine
fT4/FT4	Free thyroxine
GD	Graves' Disease
HAART	Highly active antiretroviral therapy
HDL	High-density lipoprotein
HIV	Human immunodeficiency virus
HSCT	Hematopoietic stem cell transplantation
IBM	Inclusion body myositis

IGF-1	Insulin-like growth factor 1
IGSF1	*Immunoglobulin superfamily member 1*
IRT	Immune reconstitution therapy
JCAAI	Joint Council of Allergy, Asthma & Immunology
JTFPP	Joint Task Force on Practice Parameters
LC	Liquid chromatography
LDL	Low-density lipoprotein
LDTs	Laboratory-developed tests
LT4	Levothyroxine
MMS	Mitochondrial Medicine Society
MR	Mendelian randomization
MS	Multiple sclerosis
NICE	National Institute for Health and Care Excellence
NSAID	Non-steroidal anti-inflammatories
ADEM	Acute disseminated encephalitis and encephalomyelitis
PPT	Postpartum thyroiditis
QT interval	The interval from the beginning of the QRS complex to the end of the T wave on an electrocardiogram
rhGH	Recombinant human growth hormone
RIA	Radioimmunoassay
RXR	Retinoid X receptor
SBP2	*Selenocysteine (Sec) insertion sequence-binding protein 2*
SLE	Systemic lupus erythematosus
SMFM	Society for Maternal-Fetal Medicine
T3	Triiodothyronine
T4	Thyroxine
TBG	Thyroxine-binding globulin
TC	Total cholesterol
Tg	Thyroglobulin
TG-Ab	Antithyroglobulin antibodies
THBR	Thyroid hormone binding ratio
TNs	Thyroid nodules
TPO	Thyroid peroxidase
TPOAb	Thyroid peroxidase antibody
TRAb	Thyrotropin receptor antibodies
TRAbs	T receptor antibodies
TRH	Thyrotropin-releasing hormone
TSH	Thyroid-stimulating hormone
TSHR	Thyroid-stimulating hormone receptor
TSH-R-Ab	TSH-R-stimulating antibody
TSHRAbs	Thyrotropin receptor autoantibodies
TT3	Total T3

TT4	Total T4
USPSTF	United States Preventive Services Task Force

Scientific Background

Metabolic homeostasis is regulated by the thyroid gland through production of thyroid hormones. Thyroid disease is estimated to occur in approximately 30 million Americans, much of which is undiagnosed (AACE, 2024). The thyroid gland is regulated by thyroid stimulating hormone (TSH or thyrotropin). TSH is secreted by the anterior pituitary and stimulates thyroid gland to secrete two hormones, thyroxine (T4) and triiodothyronine (T3), and TSH secretion is stimulated by thyrotropin-releasing hormone (TRH), which is distributed throughout the hypothalamus with traces found in the central nervous system and in the pituitary gland, gastrointestinal tract, pancreatic islets, and reproductive tract. Both TSH and TRH levels are controlled through a negative feedback loop by T4 and T3. Thyroid hormone production is also regulated by the extrathyroidal conversion of T4 to T3, allowing for rapid changes in tissue thyroid hormone availability (Ross, 2023b; Douglas S. Ross, 2024).

More than 99.95 percent of T4 and 99.5 percent of T3 in serum are bound to several serum proteins, including thyroxine-binding globulin (TBG), transthyretin (TTR, formerly called thyroxine-binding prealbumin [TBPA]), albumin, and lipoproteins. Since nearly all the T4 and T3 found in serum is bound, changes in serum concentration of binding proteins, namely TBG, influence the total serum T4 and T3 concentrations and the fractional metabolism of T4 and T3 (Douglas S. Ross, 2024).

Thyroid function is best assessed by measuring TSH (assuming steady-state conditions and the absence of pituitary or hypothalamic disease). However, direct measurement of all TSH and all other serum thyroid hormone levels (serum total T3 and total T4, serum free T3 (fT3) and free T4 (fT4) is still important, as it may be difficult in some patients to be certain about the state of pituitary and hypothalamic function (Ross, 2023b).

Thyroid hormones must be maintained within a carefully regulated range, as levels outside this range (both hypo- or hyperthyroid) can result in adverse clinical consequences. Hypothyroidism diagnosis depends heavily on laboratory tests because of the lack of specificity of the typical clinical manifestations. Primary hypothyroidism is characterized by high TSH and low fT4 concentrations. Subclinical hypothyroidism is defined biochemically as a patient having elevated TSH but a normal fT4 concentration and secondary (central) hypothyroidism is characterized by a patient having low serum T4 concentration but a normal serum TSH concentration. Symptoms include fatigue and weakness, cold intolerance, weight gain, cognitive dysfunction, dyspnea on exertion, hair loss, hoarseness, dry skin, edema, decreased hearing, myalgia and paresthesia, depression, menorrhagia, arthralgia, or pubertal delay (D. S. Ross, 2024). Another well-documented consequence of hypothyroidism during childhood is that of short stature, serving as presenting feature and is linked to delayed bone age, as those treated for hypothyroidism often resume their normal growth potential (Richmond & Rogol, 2024). Thus, newborns with undetected or untreated hypothyroidism will have both mental and physical developmental delay. Hypothyroidism during pregnancy increases the risk for miscarriage, preterm delivery, and pre-eclampsia (Alexander et al., 2017).

Overt hyperthyroidism refers to patients with elevated levels of fT4, fT3, or both, and subnormal TSH levels, while subclinical hyperthyroidism is defined as patients having normal T4 and T3 in the presence of subnormal TSH levels. Hyperthyroid symptoms are nonspecific, but can include tachycardia, heat intolerance, sweating, tremor, dyspnea on exertion, and weight loss. Because a number of these symptoms are so common and nonspecific, they may be subtle and unrecognized. Both hypothyroidism

and hyperthyroidism conditions rely on laboratory testing to confirm diagnosis (Ross, 2023a; D. S. Ross, 2024).

Current assays for TSH are extremely sensitive at detecting changes in thyroid homeostasis prior to changes in T4 and T3 levels. Thus, TSH assessment is the most often used initial test for thyroid function. In general, if serum TSH is normal, no further testing is needed; however, if serum TSH is high, fT4 is used to determine the degree of hypothyroidism. In contrast, if serum TSH is low, fT4 and fT3 are used to determine the degree of hyperthyroidism. If a pituitary or hypothalamic condition is suspected, both serum TSH and fT4 may be measured, and serum fT4 may be measured if symptoms of hyper- or hypothyroidism are present in a patient with normal TSH levels (Ross, 2023b). Measurement of fT4 is regarded as a better indicator of thyroid function than total T4 measurement for most situations, as it reflects the amount of available hormone. Presently, there is considerable controversy as to the appropriate upper limit of normal for serum TSH, with most labs using upper limits of approximately 4.5 to 5.0 mU/L (current "normal" range 0.4-5 mU/L) and there are debates on the cost effectiveness of screening asymptomatic patients. In addition, research has shown an age-related shift toward higher TSH concentrations in older patients (Ross, 2023b).

While thyroid nodules (TNs) are prevalent and found in up to 50% of all individuals, with most being benign, some TNs can be malignant. Evaluation of these nodules is crucial to rule out malignancy and identify those individuals requiring surgical intervention. One important laboratory test for the differentiation between a benign or malignant TN is assessment of TSH levels. In individuals with a TN, serum TSH levels that either exceed the normal range or are near the upper limit of the range are concerning, as this corresponds to an increased risk of malignancy (AlSaedi et al., 2024; Haymart et al., 2008).

Thyroiditis may be caused by an autoimmune disorder, an infection, or exposure to certain drugs or toxic chemicals which can be either acute or chronic. The evaluation of possible autoimmune thyroid disorders includes testing for the presence of thyroid antibodies. Several antibodies against thyroid antigens have been described in chronic autoimmune thyroiditis. The antigens include thyroglobulin (Tg), thyroid peroxidase (TPO) and the thyrotropin receptor. However, different levels of antibodies correspond to different conditions—for example, nearly all patients with Hashimoto's thyroiditis (chronic autoimmune hypothyroidism) have high serum concentrations of antibodies to Tg and TPO (D. S. Ross, 2024). Thyrotropin receptor antibodies (TRAb) can be classified as stimulating, blocking, or neutral. Stimulating TRAb cause Graves' disease and thyroid receptor-blocking antibodies can cause hypothyroidism. Some patients will have a mixture of both antibodies and may fluctuate between hyper- and hypothyroidism, depending on the relative titers of these stimulating and blocking antibodies. Although these antibodies are not routinely measured in evaluating thyroid function, measuring them may still be helpful for more specific goals, such as predicting progression of hypothyroidism (Ross, 2023b).

Assessment of the thyroid is particularly important for pregnant individuals. Due to the metabolic changes during pregnancy, the levels of thyroid hormones differ dramatically. In pregnant individuals, total T4 and total T3 are higher than in nonpregnant individuals, thyroxine-binding globulin nearly doubles due to the increased estrogen, and in the first trimester, TSH concentrations are reduced due to high serum human chorionic gonadotropin (hCG) levels. Thyroid physiology changes during pregnancy, therefore trimester-specific ranges for TSH and fT4 should be utilized. Unfortunately, not all commercial laboratories provide these reference ranges. As such, when trimester-specific reference ranges for fT4 are not available and fT4 levels appear discordant with TSH, total T4 measurements may be superior to fT4 (Ross, 2023c).

The effects of thyroid problems during pregnancy may be dire. Luewan et al. (2011) performed a study comparing 180 pregnant individuals with hyperthyroidism to 360 controls. The authors found that the mean gestational age and mean birth weight were significantly lower in the study group. The incidence of fetal growth restriction, low birth weight, and preterm weights were 1.3, 1.4, and 1.3 times higher, respectively, in the study group compared to the control group (Luewan et al., 2011).

An imbalance of thyroid hormones is not only harmful to pregnant individuals, but it can also negatively impact children, producing short stature. Thyroid hormone isoforms and thyroid hormones play an important role in bone development and growth, as defects associated with congenital hypothyroidism include delayed epiphyseal closure and widely spaced cranial sutures (Leung & Brent, 2016). During development, these effects extend to influence chondrocytes and growth plate cartilage in bones. Mediation of the chondrogenesis—the formation of cartilage from condensed mesenchyme tissue—by the endocrine system takes place through the action of hormones, including growth hormone, insulin-like growth factor 1 (IGF-1), androgens, glucocorticoids, and thyroid hormone. It is believed that the balance between proliferation and senescence of chondrocytes at the growth plate of bones plays a crucial role in both normal and pathologic variations of linear growth, though the pathways are unclear as of date (Leung & Brent, 2016; Richmond & Rogol, 2024).

Tests measuring levels of thyroid-related markers are widely available commercially, often as a panel. Many combinations of thyroid serum markers are available. For example, Testing.com offers thyroid tests which screen for individual thyroid hormones including TSH, fT4, and fT3 (Testing, 2024). EverlyWell offers a direct-to-consumer home-health panel testing for TSH, T3, T4, and thyroid peroxidase antibodies (EverlyWell, 2024). Other direct-to-consumer home-health panel tests include LetsGetChecked (LetsGetChecked, 2024), Paloma Health (Paloma Health, 2024), myLABBOX Thyroid Health Screening (myLABBOX, 2024), and TellmeGEN (TellmeGEN, 2023).

Common variable immunodeficiency is one of the more common antibody deficiency disorders. In one large series of primary immunodeficiency (PID) in children diagnosed over a 10-year period, CVID made up 17 of 189 total PID cases and 20 percent of the 87 cases of antibody deficiency. Most patients with CVID present after puberty, and the disorder is usually diagnosed in the second or third decade of life. However, about 25 percent of all CVID patients present in childhood or adolescence and there is an earlier peak of diagnosis occurring around eight years of age. A diagnosis of CVID before six years of age should be considered preliminary because of immunologic immaturity and the persistence of transient hypogammaglobulinemia of infancy in some children. In addition, the possible presence of a monogenic defect that causes a CVID-like disorder should be considered in children who present at a very young age. Children with failure-to-thrive should be evaluated for thyroid function and growth hormone deficiency. Growth hormone replacement therapy should be offered if deficiency is identified (Hogan & Shepherd, 2022).

Analytical Validity

The current generation of assays measuring serum TSH is a chemiluminometric assay, which have detection limits of about 0.01 mU/L. This amount is sufficiently low enough to distinguish between euthyroidism and hyperthyroidism as well as providing superior sensitivity to the prior generation of assays whose detection limits were approximately 0.1 mU/L (Ross, 2023b).

A study focusing on validating a new electrochemiluminescent assay for serum TSH, T4, and T3 found their intra-assay coefficient of variation to be under 8% for all three hormones and inter-assay coefficient of variation to be <2.9% for TSH, 2.3% for FT4, and 12.3% for T3. The correlation between this

assay and the typical ELISA or RIA assays were all at least r = .8 with many correlations near or above .9 (Kazerouni & Amirrasouli, 2012).

Serum T4 and T3 are typically measured by automated competitive binding chemiluminometric assays. Older competitive binding radioimmunoassays are still available for serum total T4. Serum total T4 and total T3 measure both bound and unbound (free) T4 and T3, respectively. A large percentage of serum T4 is bound (99.97%) to thyroxine-binding globulin (TBG), transthyretin (also called TBPA [thyroxine0binding prealbumin]), and albumin. Serum T3 is less tightly bound to TBG and TBPA but more tightly bound to albumin than T4. Normal reference ranges do vary among laboratories; however, a typical reference range for total T4 is 4.6-11.2 mcg/dL (60-145 nmol/L) and for total T3, while more variable across laboratories even than total T4, a typical reference range is ~75-195 ng/dL (~1.1-3 nmol/L) (Ross, 2023b).

The current immunoassays used to measure T3 do not always agree with other methods. For example, a study by Masika et al. (2016) compared immunoassay methods to LC/MS/MS and found that 45% of patients classified as "normal" by immunoassay were classified as "lower than 2.5th percentile" by LC/MS/MS. The authors also noted that in patients not receiving T4, 74% of their results were below the 2.5th percentile by LC/MS/MS whereas only 21% were under that mark by immunoassay. The authors speculate that this discrepancy may be due to deionidase polymorphisms, but overall conclude that because this is a significant method to diagnose thyroid issues, accuracy of T3 measurements should be paramount (Masika et al., 2016).

The measurement of reverse T3 may not be reliable. A study by Burmeister, focused on a total of 246 patients contributing 262 reverse T3 measurements, showed an inverse linear relationship between the log of TSH and reverse T3. However, Burmeister notes that hypothyroidism may cause reverse T3 to appear normal and euthyroidism may cause reverse T3 to appear low. Furthermore, it is possible that symptoms attributed to unusual reverse T3 levels are caused by hypothyroidism, despite normal TSH levels. Overall, Burmeister concludes that reverse T3 cannot differentiate between hypothyroidism and euthyroidism (Burmeister, 1995; Gomes-Lima & Burman, 2018).

Clinical Utility & Validity

Li et al. (2017) conducted a preliminary study to investigate how certain dietary supplements could affect clinical assays. They examined six healthy adult participants and 11 hormone and nonhormone analytes measured by 37 immunoassays and found that ingesting 10 mg/d of biotin for one week was associated with a potentially clinically important interference with some biotinylated assays. These immunoassays use a biotin-strepavidin binding system, so excess biotin may influence the results of assays using this system. The time at which the biotin was ingested was also a factor in the magnitude of the distortion (Li et al., 2017). Repeating a thyroid test at least two days after biotin discontinuation may be considered (Ross, 2023b).

Livingston et al. (2015) assessed the impact of T3 testing and whether T3 testing provides clinically useful information to patients who are over-treated for hypothyroidism with levothyroxine. Out of 542 patients, 33 were placed in an over-treated group, 236 were placed in a control group, and the remaining 273 did not fulfill either group. None of the patients in the over-treated group had an increased T3 and the "most discriminant" T3 level was only at 58% sensitivity and 71% specificity. The authors concluded there is no reason to measure T3 in patients with hypothyroidism on levothyroxine therapy (Livingston et al., 2015).

Yazici et al. (2016) assessed three predictors of thyroid cancer: thyrotropin (TSH), thyroglobulin (Tg), and their ratio. A study of 242 patients (134 with benign thyroid conditions, 68 with malignancy) was performed. The authors found that preoperative Tg levels were significantly lower in the malignant group (64 ng/mL vs 20 ng/mL) and that the TSH to Tg ratio was significantly higher in the malignant group, as there was no major difference in TSH between groups despite the Tg changes. However, the authors note that only fine-needle aspiration biopsy was a significant factor (Yazici et al., 2016). Autoantibodies may also play a role in the diagnosis of cancer. A study by Gholve assessing 301 samples from differentiated thyroid cancer patients (compared to 37 euthyroid controls) found the prevalence of autoantibodies in the cancer patients to be significantly higher than the controls. The authors found the prevalence of the antibodies to be 17.3% by the Immunotech kit and 16.6% by the radioassay in patients with cancer, whereas the control group was found to be only 5.4% by both methods (Gholve et al., 2017).

Thyroid antibodies play a role in autoimmune thyroiditis. A study performed by Biktagirova et al. (2016) found that 97% of patients with autoimmune thyroiditis had a high antibody-to-denatured DNA ratio compared to healthy controls. Most of these patients also had a thyroid condition (euthyroidism, hypothyroidism, hyperthyroidism) (Biktagirova et al., 2016). Another study performed by Diana investigated the prevalence of thyroid stimulating hormone receptor (TSHR) blocking antibodies (TBAb) in autoimmune thyroid disease. In total, 1079 patients with autoimmune thyroid disease (AITD) were compared to 302 controls. The authors found that about 10% of patients with AITD were positive for TBAb (82/1079). TBAb also correlated positively with TSHR binding inhibiting immunoglobulins and negatively with TSHR stimulatory antibodies. The authors concluded that TBAb was a useful and important tool to identify hypothyroidism (Diana et al., 2017).

Kluesner et al. (2018) analyzed current thyroid function test ordering practices. The authors examined 38,214 tests (encompassing TSH, fT4, TSH + fT4, fT3, Total T4, and total T3). Overall, TSH alone comprised 52.14% of tests, TSH + fT4 26.72%, fT3 alone 10.63%, fT4 alone 4.26%, and TSH + fT4 + fT3 2.74%. Free thyroid hormone testing amounted to 36% of all tests. The authors estimated the annual cost of free thyroid hormone testing to be $107,720, with savings of up to $120,000 (Kluesner et al., 2018).

Jin (2018) investigated the prevalence of subclinical hypothyroidism in obese children and its association with thyroid hormone. The study included 1,104 children and 27 of 111 (24.3%) obese children were found to have subclinical hypothyroidism, compared to 127 of 993 (12.8%) non-obese children. Body mass index was found to positively correlate with serum concentrations of TSH and negatively correlate with serum concentrations of fT4. Total cholesterol and triglyceride concentration were found to positively correlate with TSH concentrations, with fT4 negatively correlating with total cholesterol. Jin concluded that TSH is correlated with lipid profiles (Jin, 2018).

In a 2018 study, Muraresku et al. (2018)reviewed mitochondrial disease and recent advances in clinical diagnosis, management, therapeutic development, and preventative strategies. They noted that routine screening of individuals with mitochondrial diseases is imperative. Screening should include examining the "multitude of symptoms known for diabetes mellitus, adrenal insufficiency, thyroid hormone insufficiency, hearing loss, cardiac arrhythmias, and other disease related symptoms, with appropriate multi-specialist management provided." They also noted that "primary mitochondrial disease encompasses an impressive range of inherited energy deficiency disorders having highly variable molecular etiologies as well as clinical onset, severity, progression, and response to therapies of multi-system manifestations" (Muraresku et al., 2018).

Sarkar (2012) examined literature surrounding recurrent pregnancy loss in patients with thyroid dysfunction. Disturbances in thyroid function and thyroid hormone levels are common in women during their reproductive years and that dysfunction can interfere with reproductive physiology, can reduce the likelihood of pregnancy, and can adversely affect pregnancy outcome. They note that "universal screening for thyroid hormone abnormalities should be conducted in [individuals] with fetal loss or menstrual disturbances. Practitioners providing health care for women should be alert to thyroid disorders as an underlying etiology for recurrent pregnancy loss." However, universal screening for thyroid hormone abnormalities is not routinely recommended at present. In individuals capable of pregnancy and of reproductive age, hypothyroidism can be reversed by thyroxine therapy and this can improve fertility and help individuals avoid needing to use assisted reproduction technologies (Sarkar, 2012).

Korevaar et al. (2019)performed a meta-analysis focusing on thyroid function test abnormalities and thyroid autoimmunity with preterm birth. They assessed 19 cohorts encompassing 47,045 pregnant individuals and found that 1,234 of these individuals had subclinical hypothyroidism, 904 had isolated hypothyroxinemia ("decreased fT4 concentration with normal thyrotropin concentration"), 3,043 were thyroid peroxidase (TPO) antibody positive, and 2,357 had preterm birth. Risk of preterm birth was found to be higher for individuals with subclinical hypothyroidism than with euthyroidism (odds ratio = 1.29), as well as higher for individuals with isolated hypothyroxinemia (odds ratio = 1.46). The authors also found that a one standard deviation increase in maternal serum thyrotropin concentration increased risk of preterm birth by an odds ratio of 1.04. Finally, TPO antibody positive individuals were found to have a higher risk of preterm birth compared to TPO antibody negative individuals by an odds ratio of 1.33 (Korevaar et al., 2019).

In a population-based study by Kiel et al. (2020) the use of thyroid hormone measurements in ambulatory care was assessed. Measurement of serum TSH, fT3, and fT4 within the one to three years prior to the study was reported. A total of 5,552 participants were included in the analysis, with 25% (1,409/5,552) having a diagnosed thyroid disorder or treatment. Of these, 30% (1626/5552) received at least one TSH measurement and 6.8% (378/5552) received at least one thyroid ultrasound. In the study, "TSH measurement rates were 1.7 times higher than the highest reported rate (438/1000), fT4 measurement rates were within the reported range (89/1000), and fT3 was measured at a 10- fold higher rate than the highest reported (89/1000)." The study results are in accordance with current guidelines, which recommend measuring TSH levels rather than fT4/fT3 both for patients with suspected hypo- and hyperthyroidism as well as for monitoring purposes. However, the data also suggests that fT4 and fT3 were tested at the same rate, even though fT4 is recommended as sufficient to distinguish between overt and subclinical hypothyroidism. Despite overuse of thyroid hormone testing, there is possible underuse in patients with diagnosed thyroid disorders who are taking thyroid medication. In the study, 40% did not receive a monitoring TSH test within one year, and 16% did not receive a TSH test within three years. The authors suggest that "Given the frequency of patients with thyroid disorders, diagnostic and monitoring tests should be used rationally with regard to costs. TSH levels should be monitored regularly in patients on thyroid medication" (Kiel et al., 2020).

In 2021, Degrandi et al. (2021) examined the prevalence of thyroid autoimmunity in children with developmental dyslexia. Serum TSH, fT3, and fT4 were measured and thyroid autoimmunity was evaluated by measuring TPOAbs and antithyroglobulin antibodies (TG-Abs). The authors also performed thyroid ultrasonography in the subjects with developmental dyslexia. The study enrolled 51 subjects with developmental dyslexia (M : F = 39 : 12, mean age 12.4 ± 9 years) and 34 controls (M : F = 24 : 10, mean age 10.8 ± 4 years) and found a significant increase in TPOAb positivity in subjects as compared to controls (60.8% vs 2.9%, $p<0.001$) but no significant change in TG-Ab positivity (16% vs 5.8%).

Additionally, in the subjects with developmental dyslexia who received ultrasonography (49 of 51 subjects), 60% of them had a thyroiditis pattern. Overall, this study showed a high prevalence of thyroid autoimmunity in children with developmental dyslexia and while further research is needed to confirm these initial findings, these results may change the approach to developmental dyslexia and eventually lead to a systematic determination of thyroid autoimmunity in affected children (Degrandi et al., 2021).

Wang et al. (2021) examined the association between thyroid function and serum lipid metabolism, utilizing a genetic analysis termed Mendelian randomization (MR). While thyroid dysfunction is known to be associated with cardiovascular disease, the role of thyroid function in lipid metabolism is still partly unknown. "The MR approach uses a genetic variant as the instrumental variable in epidemiological studies to mimic a randomized controlled trial" and for this study, the authors performed a two-sample MR to assess the causal association, using summary statistics from the Atrial Fibrillation Genetics Consortium (n = 537,409) and the Global Lipids Genetics Consortium (n = 188,577). TSH, fT3 and fT4 levels, the fT3:fT4 ratio, and the concentration of TPOAb were all used to get a clinical measurement of thyroid function. Serum lipid metabolism traits included total cholesterol (TC) and triglycerides, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) levels. To assess the association between thyroid function and serum lipid metabolism, the MR estimate and MR inverse variance-weighted method were used. The authors found that increased TSH levels were significantly associated with higher TC and LDL levels, as was the fT3:fT4 ratio. However, they observed no significant differences between genetically predicted fT4 and TPOAb and serum lipids. They concluded that their results suggest an association between thyroid function and serum lipid metabolism, "highlighting the importance of the pituitary-thyroid-cardiac axis in dyslipidemia susceptibility" (Wang et al., 2021).

Toloza et al. (2022) performed a systematic review and meta-analyses of data collected from pregnant patients (excluding pre-existing thyroid disease and multifetal pregnancies) to analyze the primary outcomes of gestational hypertension and pre-eclampsia; data was taken from cohort studies that included maternal concentrations of TSH, FT4, and TPO antibodies as well as data regarding maternal gestational hypertension, pre-eclampsia, or both. The study comprised 46,528 pregnant individuals, of which 39,826 individuals had enough data to be classified by thyroid function status. Individuals who had subclinical hypothyroidism made up 3.2% of the cohort (1,275 individuals). After analyses, a total of 933 individuals had isolated hypothyroxinemia, 619 had subclinical hyperthyroidism, and 337 had overt hyperthyroidism. The authors concluded that "compared with euthyroidism, subclinical hypothyroidism was associated with a higher risk of pre-eclampsia…In a continuous analysis, both a higher and a lower TSH concentration were associated with a higher risk of pre-eclampsia" (Toloza et al., 2022).

Guidelines and Recommendations

United States Preventive Services Task Force (USPSTF)

The USPSTF states that "current evidence is insufficient to assess the balance of benefits and harms of screening for thyroid dysfunction in non-pregnant, asymptomatic adults" (Rugge et al., 2015). As such, USPSTF recommends against screening for thyroid cancer in asymptomatic adults (USPSTF, 2017).

American College of Obstetricians and Gynecologists (ACOG)

The ACOG published an updated guideline regarding Thyroid Disease in Pregnancy in June 2020. The following recommendations are based on good and consistent scientific evidence (Level A):

- "Universal screening for thyroid disease in pregnancy is not recommended because identification and treatment of maternal subclinical hypothyroidism has not been shown to result in improved pregnancy outcomes and neurocognitive function in offspring.
- If indicated, the first-line screening test to assess thyroid status should be measurement of the TSH level.
- The TSH level should be monitored in pregnant [individuals] being treated for hypothyroidism, and the dose of levothyroxine should be adjusted accordingly with a goal TSH level between the lower limit of the reference range and 2.5 milliunits/L. Thyroid-stimulating hormone typically is evaluated every 4–6 weeks while adjusting medications.
- Pregnant [individuals] with overt hypothyroidism should be treated with adequate thyroid hormone replacement to minimize the risk of adverse outcomes.
- The level of free T4 should be monitored in pregnant [individuals] being treated for hyperthyroidism, and the dose of antithyroid drug (thioamide) should be adjusted accordingly to achieve a free T4 at the upper end of the normal pregnancy range. Among women who also have T3 thyrotoxicosis, total T3 should be monitored with a goal level at the upper end of normal pregnancy range.
- Pregnant [individuals] with overt hyperthyroidism should be treated with antithyroid drugs (thioamides)."

The following recommendation is based on limited or inconsistent scientific evidence (Level B):

- "Either propylthiouracil or methimazole, both thioamides, can be used to treat pregnant [individuals] with overt hyperthyroidism. The choice of medication is dependent on trimester of pregnancy, response to prior therapy, and whether the thyrotoxicosis is predominantly T4 or T3."

The following recommendations are based primarily on consensus and expert opinion (Level C):

- "Indicated testing of thyroid function should be performed in women with a personal or family history of thyroid disease, type 1 diabetes mellitus, or clinical suspicion of thyroid disease.
- Measurements of thyroid function are not recommended in patients with hyperemesis gravidarum unless other signs of overt hyperthyroidism are evident."

Other miscellaneous, relevant comments from ACOG include:

- "Indicated testing of thyroid function should be performed in women with a personal or family history of thyroid disease, type 1 diabetes mellitus, or clinical suspicion of thyroid disease… In a pregnant woman with a significant goiter or with distinct thyroid nodules, thyroid function studies are appropriate…"
- "In cases of suspected hyperthyroidism, total T3 also is measured...Total T3 is used preferentially over free T3 because assays for estimating free T3 are less robust than those measuring free T4…"
- "Routine testing for antithyroid peroxidase antibodies in women who are euthyroid (eg, no history of thyroid disease and normal thyroid function tests) is not recommended because thyroid hormone replacement for antithyroid peroxidase antibodies alone has not been found to improve pregnancy outcomes… Identification of thyroid antibodies including thyroid receptor antibodies and thyroid stimulating immunoglobulin in women with Graves [*sic*] disease may establish those at an increased risk for fetal or neonatal hyperthyroidism" (ACOG, 2020).

American Thyroid Association (ATA) and American Association of Clinical Endocrinologists (AACE)

The ATA and AACE support TSH testing for individuals with the following conditions: adrenal insufficiency, alopecia, unexplained anemia, unexplained cardiac dysrhythmia, skin texture changes, congestive heart failure, constipation, dementia, type 1 diabetes, dysmenorrhea, hypercholesterolemia, hypertension, mixed hyperlipidemia, malaise and fatigue, unexplained myopathy, prolonged QT interval, vitiligo, or weight gain. The guidelines also recommend assessing serum fT4 instead of total T4 to diagnose hypothyroidism except with pregnant patients.

The ATA and AACE also provide recommendations for thyroid antibody testing including:

- "Anti-thyroid peroxidase antibody (TPOAb) measurements should be considered when evaluating patients with subclinical hypothyroidism."
- TPOAb measurement should be considered in evaluation of patients with recurrent miscarriage, regardless of fertility.
- "Measurement of [Thyrotropin receptor autoantibodies] TSHRAbs should be considered in hypothyroid pregnant patients with history of Graves' disease if treated with radioactive iodine or thyroidectomy before pregnancy. This should be done either at 20-26 weeks of gestation or during the first trimester and if they are elevated, again at 20-26 weeks of gestation" (Garber et al., 2012).

The guidelines recommend against testing serum T3 or fT3, as well as use of clinical scoring systems to diagnose hypothyroidism. In patients with central hypothyroidism, the guidelines recommend assessing either fT4 or its index and to avoid testing for TSH (Garber et al., 2012).

American Thyroid Association (ATA)

Diagnosis and Management of Thyroid Disease During Pregnancy and Postpartum

In 2011, the ATA stated that it does not recommend "universal" TSH or free T4 screening of pregnant women or during the preconception period. It also included the following recommendations:

Thyroid Function Tests in Pregnancy: Trimester-specific reference ranges for TSH, as defined in populations with optimal iodine intake, should be applied. The ATA recommends these reference ranges: first trimester, 0.1–2.5 mIU/L; second trimester, 0.2–3.0 mIU/L; third trimester, 0.3–3.0 mIU/L.

The best method to assess serum fT4 during pregnancy is measurement of T4 in the dialysate or ultrafiltrate of serum samples employing LC/MS/MS. If this is not available, clinicians should use the next best method available. However, serum TSH is a more accurate indication of thyroid status in pregnancy than any of these alternative methods. Method-specific and trimester-specific reference ranges of serum fT4 are required.

Thyrotoxicosis in Pregnancy: If the first trimester serum TSH appears low (<0.1 mIU/L), a history and physical examination are indicated. fT4 measurements should be obtained in all patients. Measurement of serum total T3 (TT3) and thyrotropin receptor antibodies (TRAb) may be helpful in establishing a diagnosis of hyperthyroidism. If the patient has a history of Graves' disease, a maternal serum sample of TRAb should be obtained at 20–24 weeks gestation.

Thyroid Nodules and Thyroid Cancer: Treatment of thyroid nodules during pregnancy will depend on risk assessment. However, all women should have the following: a complete history and clinical examination, serum TSH testing, and ultrasound of the neck. Thyroid hormone therapy may be considered in pregnant women who have deferred surgery for well-differentiated thyroid carcinoma until postpartum.

The goal of levothyroxine (LT4) therapy is a serum TSH level of 0.1–1.5 mIU/L. Furthermore, a preconception TSH goal (determined by risk assessment) should be set in women with differentiated thyroid cancer. This goal should be maintained during pregnancy with monitoring every four weeks until 16-20 weeks of gestation followed by once between 26 and 32 weeks of gestation.

Postpartum Thyroiditis (PPT): Women with postpartum depression should have TSH, fT4, and TPOAb tests performed. Women who are symptomatic with hypothyroidism in PPT should either have their TSH level retested in four to eight weeks or be started on LT4 in certain situations (such as if symptoms are severe). Women who are asymptomatic with hypothyroidism in PPT should have their TSH level retested in four to eight weeks. Finally, women with a history of PPT should have an annual TSH test to evaluate for permanent hypothyroidism.

Thyroid Function Screening in Pregnancy: There is insufficient evidence regarding universal TSH screening at the first trimester visit. Serum TSH values should be obtained early in pregnancy in the following women at high risk for overt hypothyroidism:

- History of thyroid dysfunction or prior thyroid surgery
- Age >30 years
- Symptoms of thyroid dysfunction or the presence of goiter
- TPOAb positivity
- Type 1 diabetes or other autoimmune disorders
- History of miscarriage or preterm delivery
- History of head or neck radiation
- Family history of thyroid dysfunction
- Morbid obesity (BMI ≥40 kg/m^2)
- Use of amiodarone or lithium, or recent administration of iodinated radiologic contrast
- Infertility
- Residing in an area of known moderate to severe iodine insufficiency (Stagnaro-Green et al., 2011)

The ATA published an update in 2017 for thyroid function testing during pregnancy. Recommendations include:

- "The accuracy of serum FT4 measurement by the indirect analog immunoassays is influenced by pregnancy and also varies significantly by manufacturer. If measured in pregnant women, assay method-specific and trimester-specific pregnancy reference ranges should be applied."
- "In lieu of measuring FT4, TT4 measurement (with a pregnancy-adjusted reference range) is a highly reliable means of estimating hormone concentration during the last part of pregnancy. Accurate estimation of the FT4 concentrations can also be done by calculating a FT4 index."
- Total T4 measurement (with a pregnancy-adjusted reference range) is reliable for estimating concentration late in pregnancy. A free thyroxine index can also estimate fT4 well.
- Euthyroid and TPO or Tg antibody positive pregnant women should have serum TSH concentration measured at the start of pregnancy and every 4 weeks through mid-pregnancy.
- All women seeking care for infertility are recommended to have serum TSH levels measured.
- Pregnant women with TSH concentrations >2.5 mU/L should be evaluated for TPO antibodies.
- Women with hypothyroidism or those at risk for hypothyroidism (e.g. patients who are euthyroid but TPO or TGAb positive) should be monitored with a serum TSH measurement every 4 weeks until mid-gestation, and at least once near 30 weeks.

- "When a suppressed serum TSH is detected in the first trimester (TSH less than the reference range), a medical history, physical examination, and measurement of maternal serum FT4 or TT4 concentrations should be performed. Measurement of TRAb and maternal TT3 may prove helpful in clarifying the etiology of thyrotoxicosis"
- In women being treated with antithyroid drugs [ATDs] in pregnancy, fT4/TT4 and TSH should be monitored every 4 weeks.
- "All patients with depression, including postpartum depression, should be screened for thyroid dysfunction."
- "Evaluation of serum TSH concentration is recommended for all women seeking care for infertility."
- "If the patient has a past history of GD [Graves Disease] treated with ablation (radioiodine or surgery), a maternal serum determination of TRAb is recommended at initial thyroid function testing during early pregnancy. If maternal TRAb concentration is elevated in early pregnancy, repeat testing should occur at weeks 18–22.
- "If the patient requires treatment with ATDs for GD through mid-pregnancy, a repeat determination of TRAb is again recommended at weeks 18–22. If elevated TRAb is detected at weeks 18–22 or the mother is taking ATD in the third trimester, a TRAb measurement should again be performed in late pregnancy (weeks 30–34) to evaluate the need for neonatal and postnatal monitoring."
- "The utility of measuring calcitonin in pregnant women with thyroid nodules is unknown. The task force cannot recommend for or against routine measurement of serum calcitonin in pregnant women with thyroid nodules."
- "All newborns should be screened for hypothyroidism by blood spot analysis typically 2–5 days after birth."
- "Following the resolution of the thyrotoxic phase of PPT, serum TSH should be measured in approximately 4–8 weeks (or if new symptoms develop) to screen for the hypothyroid phase."
- "Women with a prior history of PPT should have TSH testing annually to evaluate for the development of permanent hypothyroidism"
- "There is insufficient evidence to recommend for or against universal screening for abnormal TSH concentrations in early pregnancy.
- "There is insufficient evidence to recommend for or against universal screening for abnormal TSH concentrations preconception, with the exception of women planning assisted reproduction or those known to have TPOAb positivity"
- "Universal screening to detect low FT4 concentrations in pregnant women is not recommended" (Alexander et al., 2017).

The guideline also lists certain populations of pregnant women that should have serum TSH measured "as soon as pregnancy is confirmed" due to presence of risk factors of thyroid disease. These risk factors include "history of thyroid dysfunction, symptoms or signs of thyroid dysfunction, presence of a goiter, and known thyroid antibody positivity…age >30 years, history of diabetes mellitus type 1, or other autoimmune disorders, history of pregnancy loss, preterm delivery or infertility, history of head or neck radiation or prior thyroid surgery, family history of autoimmune thyroid disease or thyroid dysfunction, morbid obesity, use of amiodarone, lithium, or recent administration of iodinated radiologic contrast, two or more prior pregnancies, and residing in area of moderate to severe iodine deficiency" (Alexander et al., 2017).

The American Thyroid Association recommends that "the appropriate management of abnormal maternal thyroid tests attributable to gestational transient thyrotoxicosis and/or hyperemesis gravidarum includes supportive therapy, management of dehydration, and hospitalization if needed.

Antithyroid drugs are not recommended, though β-blockers may be considered. In women being treated with antithyroid drugs in pregnancy, FT4/TT4 and thyroid hormone secretion should be monitored approximately every 4 weeks. Antithyroid medication during pregnancy should be administered at the lowest effective dose of MMI or PTU, targeting maternal serum FT4/TT4 at the upper limit or moderately above the reference range. A combination regimen of LT4 and antithyroid drugs should not be used in pregnancy, except in the rare situation of isolated fetal hyperthyroidism" (Alexander et al., 2017).

Task Force on Thyroid Hormone Replacement for Hypothyroidism Treatment (2014)

The ATA recommended LT4 as the primary treatment of choice for hypothyroidism due to overall efficacy, low cost, and lack of side effects. The ATA also states that great care should be taken to monitor dose diligently especially in pregnant women, as excessive LT4 can have dangerous side effects (Jonklaas et al., 2014).

Guidelines for Diagnosis and Management of Hyperthyroidism and Other Causes of Thyrotoxicosis (2016)

The ATA recommends that the cause of the thyrotoxicosis should be determined. Initial diagnostic tests include measurement of TRAb, radioactive iodine uptake, or measurement of thyroidal blood flow on ultrasonography. The guidelines also note that serum TSH is the most accurate and should be the first screening test done, but if thyrotoxicosis is suspected, it is helpful to test fT4 and T3.

The ATA recommends treatment of subclinical hyperthyroidism (persistent TSH <0.1 mU/L) for the high-risk populations such as those with cardiac risk factors or those older than 65. Treatment of asymptomatic and otherwise healthy individuals may be considered. The ATA also recommends testing TRAb in pregnant women with unknown hyperthyroidism. A diagnosis of hyperthyroidism should made with the serum TSH values and trimester-specific reference ranges for T4 and T3 (Ross et al., 2016).

American Academy of Family Physicians (AAFP)

The AAFP has recommended this diagnostic workup for hyperthyroidism: "measuring TSH, free (T4), and total T3 levels to determine the presence and severity of the condition, as well as radioactive iodine uptake and scan of the thyroid to determine the cause." The level of this evidence is C which is a consensus, disease-oriented evidence, usual practice, expert opinion, or case series (Kravets, 2016). The AAFP also recommends using TSH testing to diagnose primary hypothyroidism (Level C) (Gaitonde et al., 2012).

In the case of subclinical thyroid disease, the AAFP recommends that "Physicians should not routinely screen for subclinical thyroid disease" (Donangelo & Suh, 2017). Moreover, the AAFP reaffirms its support for the USPSTF stance on thyroid dysfunction, stating that there is no evidence that population screening is beneficial and that "Screening for thyroid dysfunction in nonpregnant, asymptomatic individuals has uncertain risks and benefits" as there has been a dearth of studies comparing the benefits of harms of screening against no screening (AAFP, 2024; Wilson et al., 2021).

The American Family Physician recommends "the term failure to thrive should be used as a clinical finding and not as a diagnosis. Recognition depends on reliable and valid measurements over time; therefore, serial measurements of weight and height must be accurately obtained and charted on an appropriate reference scale. No standard set of laboratory tests is recommended for failure to thrive. A thorough history and physical examination may be all that is indicated to initiate treatment. If used, reasonable initial laboratory testing includes complete blood count, urinalysis, electrolyte measurement,

thyroid tests, and testing for celiac disease. Specific testing for cystic fibrosis, food allergies, human immunodeficiency virus infection, or tuberculosis may be indicated depending on the presentation. Additional testing should be specific for a suspected diagnosis based on history and physical examination findings" (Homan, 2016).

American Academy of Pediatrics - Section on Endocrinology (AAP)

The American Academy of Pediatrics recommends against routinely measuring thyroid function or insulin levels in obese children, as well as screening healthy children for thyroid problems (AAP, 2017).

Australian Journal of General Practice (AJGP)

According to the Australian Journal of General Practice, "Thyroid stimulating hormone (TSH) should be checked:

- when screening for thyroid hormone excess or deficiency on the basis of symptoms or risk factors
- when goitre or thyroid nodules are identified
- when monitoring uncomplicated thyroxine replacement therapy, with a minimum interval of 4–6 weeks following a dose change to allow achievement of a steady state, and annually when stable
- prior to, and early in the first trimester of, pregnancy in women treated with levothyroxine or those with risk factors for thyroid dysfunction."

Moreover, "An elevated TSH level should be investigated in the following ways:

- If TSH is high, check TSH with free T4 (FT4)
    - elevated TSH with FT4 below the reference range diagnoses primary hypothyroidism
    - elevated TSH with FT4 within the reference range diagnoses mild or subclinical hypothyroidism.
- A mildly raised TSH will often resolve without treatment; therefore, thyroid function tests should generally be repeated at least once after 1–3 months before further investigation or treatment.
- Thyroid ultrasonography and thyroid scintigraphy should not be performed for uncomplicated hypothyroidism without a palpable nodule."

The AJGP also outlines when a suppressed TSH level be investigated:

- "if TSH is low, check FT4 and FT3
    - FT4 and/or FT3 above the reference range diagnoses primary hyperthyroidism
    - a mildly low TSH (0.1–0.5 mIU/L) with normal free thyroid hormones suggests mild or subclinical hyperthyroidism, non-thyroidal illness or interference from other medications
- positive TSH receptor antibodies (TRAb; or thyroid stimulating immunoglobulins [TSI]) support a diagnosis of Graves' disease
- thyroid scintigraphy should be performed to distinguish between Graves' disease, toxic nodules and thyroiditis if the TRAb test is negative or there is diagnostic uncertainty
- thyroid ultrasonography is generally unhelpful in determining the cause of hyperthyroidism."

"It is important to:

- check TSH, FT4 and FT3 to evaluate thyroid function in settings where TSH alone may be unreliable, such as
    - suspected pituitary or hypothalamic disease

    - suspected assay interference
    - rapidly changing thyroid function
- check FT4 (not TSH) to monitor and adjust levothyroxine replacement in patients with central hypothyroidism due to pituitary or hypothalamic disease
- note that measurement of reverse T3 is not recommended for the investigation of thyroid dysfunction" (Croker et al., 2021).

The Australian Journal of General Practice recommends that "couples with two of more pregnancy losses should have thyroid antibody and function testing performed. Abnormal results should be managed by a specialized clinic. There is evidence that suggests hypothyroidism and even subclinical hypothyroidism is associated with recurrent pregnancy loss. All guidelines recommend testing for thyroid-stimulating hormone (TSH) levels, but there is contention about what is considered a 'normal' TSH. Current guidelines suggest treating all women with overt hypothyroidism, considering treatment of subclinical hypothyroidism, and not treating euthyroid patients with recurrent pregnancy loss who test positive for thyroid antibodies" (Li & Marren, 2018).

Joint Task Force on Practice Parameters (JTFPP) of the Academy of Allergy, Asthma & Immunology (AAAAI); the American Academy of Allergy, Asthma & Immunology (AAAAI); and the Joint Council of Allergy, Asthma & Immunology (JCAAI)

The JTFPP within their guidelines concerning the diagnosis and management of acute and chronic urticaria state, "Targeted laboratory testing based on history or physical examination findings is appropriate, and limited laboratory testing can be obtained. Limited laboratory testing includes a CBC with differential, sedimentation rate, and/or C-reactive protein, liver enzyme, and thyroid-stimulating hormone (TSH) measurement… Targeted laboratory testing based on history and/or physical examination (eg, obtaining TSH in a patient with weight gain, heat/cold intolerance, and thyromegaly) is recommended" (Bernstein et al., 2014).

American Society for Clinical Pathology (ASCP)

The American Society for Clinical Pathology recommends against ordering multiple tests for an initial evaluation for a patient with a suspected thyroid condition. The ASCP recommends starting with TSH and proceeding from that result. Any diagnosis made by the physician should be confirmed with free thyroxine (T4) testing. They also recommends avoiding TSH screening in annual well-visits for asymptomatic adults, regardless of age, as there is no evidence to support that routine screening improves patient care. ASCP advises TSH screening when patients are considered at-risk or demonstrate subtle or direct signs of thyroid dysfunction upon physical evaluation (ASCP, 2020).

Endocrine Society (ES)

The Endocrine Society recommends against testing for total or free T3 when evaluating LT4 dose in hypothyroid patients. They also recommend against ordering routine ultrasounds for patients without palpable abnormalities of the thyroid. While routine thyroid ultrasounds should not be ordered without palpable abnormalities, thyroid vascularity assessments may be performed by color flow Doppler in patients who show overt hyperthyroidism evidenced by elevated free T4 and T3 and lower TSH values; color flow Doppler (a noninvasive ultrasound test) may help diagnose Graves' hyperthyroidism and toxic nodular goiter from destructive thyroiditis (Endocrine Society, 2022).

European Thyroid Association (ETA)

Management of Thyroid Dysfunction following Immune Reconstitution Therapy (IRT) (Muller et al., 2019)

This guideline discusses IRT in the context of three clinical situations; "alemtuzumab (Lemtrada) treatment for active relapsing remitting multiple sclerosis (MS); (2) after treatment of human immunodeficiency virus (HIV) infected patients with highly active antiretroviral therapy (HAART); (3) following allogeneic bone marrow transplantation (BMT) or hematopoietic stem cell transplantation (HSCT)."

The ETA recommends measuring TSH in all subjects before IRT. If TSH is abnormal, fT4 and fT3 are recommended to be measured.

Routine measurement of TPOAb or TRAb is not recommended before IRT.

TSH measurement is recommended post-IRT, and fT4 may also be routinely measured. If TSH is low (0.10–0.39 mU/L), another test is recommended within one month. If TSH is elevated, a repeat TSH test is recommended, along with fT4. If TSH is "suppressed" (<0.10 mU/L), TSH, fT4, and fT3 are recommended to be tested.

Following alemtuzumab, the ETA recommends "biochemical follow-up" with TSH testing every three months. Routine TSH monitoring is not recommended following HAART treatment in HIV patients, although TSH measurement should be performed if thyroid dysfunction is suspected.

Routine measurement of thyroid autoantibodies is not recommended in euthyroid patients during surveillance.

The ETA recommends "routine 3 monthly measuring of thyroid function to be continued for 4 years following the last alemtuzumab treatment" (Muller et al., 2019).

Thyroid Disorders Prior to and during Assisted Reproduction

The ETA recommends women of subfertile couples ("subfertile" is defined by the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse) should be screened routinely for the presence of thyroid disorders. The ETA notes that severe thyroid dysfunction is linked to menstrual disorders as well as subfertility. In a meta-analyses that included mostly women with TSH levels above 4.0 mIU/L, treatment with levothyroxine was effective at increasing live birth rates (Poppe et al., 2021).

Management of Graves' Hyperthyroidism

The ETA notes measurement of TSH-R-stimulating antibody (TSH-R-Ab) as a "sensitive and specific" tool for rapid and accurate differential diagnosis for Graves' hypothyroidism. Differentiation of TSH-R-Ab is also "helpful and predictive" in Graves' patients during pregnancy/postpartum, as well as extrathyroidal manifestations.

The ETA also remarks that measurement of TSH-R-Ab levels prior to stopping antithyroid drug treatment (ATD).

For pregnant patients, maternal fT4 and TSH should be measured every two weeks after initiation of therapy, every four weeks after achieving the target value. All patients with history of autoimmune thyroid disease should have their TSH-R-Ab levels tested at first presentation with pregnancy, and if

maternal TSH-R-Ab remains high (> three times normal cutoff), monitoring the fetus for thyroid dysfunction throughout pregnancy is recommended (Kahaly et al., 2018).

In 2022, the ETA published guidelines for the management of GD in pediatric patients. Hyperthyroidism caused by GD is relatively rare in children and treatment options for pediatric patients are the same as those available to adults (ATD, radioactive iodine (RAI), or thyroid surgery). However, the risks and benefits of each modality are different in pediatric patients than they are in adult patients. The ETA recommends that "clinicians should be alert that GD may present with behavioral changes or declining academic performance in children. Measurement of serum TSH receptor antibodies is recommended for all pediatric patients with hyperthyroidism. Management recommendations include the first-line use of a prolonged course of methimazole/carbimazole ATD treatment (three years or more), a preference for dose titration instead of block and replace ATD, and to avoid propylthiouracil use. Where definitive treatment is required either total thyroidectomy or RAI is recommended, aiming for complete thyroid ablation with a personalized RAI activity. We recommend avoiding RAI in children under 10 years of age but favor surgery in patients with large goiter. Pediatric endocrinologists should be involved in all cases" (Mooij et al., 2022).

Diagnosis and Management of Central Hypothyroidism

The ETA also published a guideline regarding central hypothyroidism (CeH). Below are the relevant recommendations:

- "We recommend that the diagnosis of CeH should be considered in every subject with low serum concentrations of FT4 and low or normal TSH on a screening examination.
- We recommend that the diagnosis of CeH should be considered in neonates and children with clinical manifestations of congenital hypothyroidism but low or normal neonatal TSH screening.
- We suggest that the diagnosis of CeH should be considered in patients with a low serum concentration of FT4 and slight TSH elevations (< 10 mU/L, or inappropriately lower than expected on the basis of the hypothyroid state).
- We recommend screening for CeH all children with a familial history of CeH and/or failure to thrive, developmental delay, GH deficiency, delayed or precocious puberty, or other hypothalamic-pituitary defects or lesions.
- We recommend that CeH due to *immunoglobulin superfamily member 1* (*IGSF1)* defect should be ruled out in adolescents or adult patients with macroorchidism.
- We recommend screening for CeH all patients with a personal or familial history of hypothalamic-pituitary lesions or diseases, moderate to severe head trauma, stroke, previous cranial irradiation, hemochromatosis or iron overload, in particular when hypothyroid manifestations are present.
- We recommend screening for CeH all patients with hypothyroid manifestations associated with clinical findings pointing to a hypothalamic-pituitary disease (e.g., hyperprolactinemia, acromegalic features, diabetes insipidus, recurrent headaches, visual field defects), newborns with hypotonia and/or prolonged jaundice, and/or signs of congenital hypopituitarism (e.g., micropenis with undescended testes), as well as children with developmental delay.
- We recommend that the onset of CeH should be evaluated in patients with hypothalamic/pituitary disease after the start of treatment with recombinant human growth hormone (rhGH) or estrogen.
- We recommend that the onset of CeH should be evaluated in patients on treatments with ligands of the retinoid X receptor (RXR), ipilimumab (or other checkpoint inhibitors), or mitotane."

Regarding diagnosis of CeH, the guideline recommends the following:

- "We recommend the combined determination of serum FT4 and TSH in order to evaluate the presence of CeH.
- We recommend that CeH diagnosis should be confirmed by the combined findings of serum FT4 concentrations below the lower limit of the normal range and inappropriately low/normal TSH concentrations on at least two separate determinations, and after exclusion of the conditions reported in Table 3.
- The isolated finding of low FT3 or total T3 concentrations is not indicative of CeH, but rather of nonthyroidal illness or deiodination defects (e.g., *selenocysteine (Sec) insertion sequence-binding protein 2 (SBP2)* gene defect).
- In patients under follow-up for hypothalamic-pituitary disease, FT4 and TSH should be monitored during childhood at least biannually and later on a yearly basis, and we suggest that CeH diagnosis should be considered when serum FT4 falls in the lower quartile of the normal range, in particular when a FT4 decrease > 20% of previous values is seen (provided that the variables are measured by the same assay) despite a low or normal TSH.
- We suggest that the diagnosis of mild CeH (borderline low FT4, with inappropriately low TSH) should be supported by a combination of several other findings summarized in Table 4 (the relative application and importance of these tests and findings may vary in different settings).

In their 2023 clinical practice guidelines for thyroid nodule management, the ETA recommends that:

- "Initial evaluation should include personal and family history, physical evaluation, thyroid function testing, and neck US assessment (*Ungraded good practice statement. Agreement: 9/9 (100%); round: 1*)
- Consider the use of a disease-specific patient-reported outcome (PRO) measure for evaluation of symptomatology (*Strength of recommendation: 1; quality of evidence: ØØØO. Agreement: 8/9 (88.9%); round: 1*)" (Durante et al., 2023).

National Institute for Health and Care Excellence (NICE)

Thyroid Disease: Assessment and Management

NICE states to "consider" thyroid dysfunction tests for adults, children, and "young people" for the following indications:

- "a clinical suspicion of thyroid disease"
- New-onset atrial fibrillation
- Type 1 diabetes or other autoimmune disease
- Depression or unexplained anxiety
- For children and young people, consider tests for abnormal growths or unexplained change in behavior or school performance

NICE states not to test for thyroid dysfunction if a patient only has type 2 diabetes or if the patient has an unrelated acute illness.

If secondary thyroid disease (pituitary disease) is not suspected, NICE states to "consider" measuring TSH. If TSH is "above reference range", measure fT4 in same sample; if TSH is "below reference range", measure fT4 and fT3 in same sample.

Measurement of both TSH and fT4 is to be considered for children or young people or if secondary thyroid dysfunction is suspected in adults. If TSH is below the reference range, fT3 should be measured. If symptoms in the above situations worsen, repeat the algorithms.

In a 2023 update, the NICE offered some additional guidance on testing when thyroid dysfunction is suspected, namely to

"1.2.8 Consider measuring thyroid-stimulating hormone (TSH) alone for adults when secondary thyroid dysfunction (pituitary disease) is not suspected. Then:

- if the TSH is above the reference range, measure free thyroxine (FT4) in the same sample
- if the TSH is below the reference range, measure FT4 and free tri-iodothyronine (FT3) in the same sample.

1.2.9 Consider measuring both TSH and FT4 for:

- adults when secondary thyroid dysfunction (pituitary disease) is suspected
- children and young people.

  If the TSH is below the reference range, measure FT3 in the same sample.

1.2.10 Consider repeating the tests for thyroid dysfunction in recommendations 1.2.8 or 1.2.9 if symptoms worsen or new symptoms develop (but no sooner than 6 weeks from the most recent test).

1.2.11 Ask adults, children and young people with suspected thyroid dysfunction about their biotin intake because a high consumption of biotin from dietary supplements may lead to falsely high or low test results" (NICE, 2023).

For adults with TSH levels above the reference range, TPOAb measurement may be considered. However, this testing should not be repeated. This applies to primary and subclinical hypothyroidism.

For children and young people, this measurement should be repeated when they become adults.

"For adults who are taking levothyroxine for primary hypothyroidism, consider measuring TSH every 3 months until the level has stabilised (2 similar measurements within the reference range 3 months apart), and then once a year." For adults with hypothyroidism symptoms after starting levothyroxine, consider measuring fT4 along with TSH.

For children ages two and over and young people taking levothyroxine for primary hypothyroidism, consider measuring fT4 and TSH "every 6 to 12 weeks until the TSH level has stabilised (2 similar measurements within the reference range 3 months apart), then every 4 to 6 months until after puberty, then once a year."

For children under two, consider measuring fT4 and TSH "every 4 to 8 weeks until the TSH level has stabilised (2 similar measurements within the reference range 2 months apart), then every 2 to 3 months during the first year of life, and every 3 to 4 months during the second year of life."

For adults with untreated subclinical hypothyroidism or adults that have stopped treatment, consider measuring TSH and fT4 once a year if they are symptomatic, or once every two to three years if they are asymptomatic.

NICE states to consider measuring fT4 and TSH for children two and over with untreated subclinical hypothyroidism and TSH <10 mIU/liter at the following intervals: "every 3 to 6 months if they have features suggesting underlying thyroid disease, such as thyroid dysgenesis (an underdeveloped thyroid gland) or raised levels of thyroid autoantibodies, or every 6 to 12 months if they have no features suggesting underlying thyroid disease."

Furthermore, "Every 1-3 months for children ages 28 days-2 years with untreated subclinical hypothyroidism." TSH measurements may be stopped in children and young people if TSH has stabilized (defined as "2 similar measurements within the reference range 3 to 6 months apart") and there are no underlying features suggesting thyroid disease.

Differentiating between thyrotoxicosis with hyperthyroidism and thyrotoxicosis without hyperthyroidism may be performed by measuring TSH receptor antibodies (TRAbs). In children and young people, measuring TPOAbs and TRAbs may be done to differentiate.

After radioactive iodine treatment, consider measuring fT3, fT4, and TSH every six weeks for the first six months, until TSH is within reference range.

"For adults, children and young people with TSH in the reference range 6 months after radioactive iodine treatment, consider measuring TSH (with cascading) at 9 months and 12 months after treatment."

"For adults, children and young people with TSH in the reference range 12 months after radioactive iodine treatment, consider measuring TSH (with cascading) every 6 months unless they develop hypothyroidism."

For patients taking antithyroid drugs for hyperthyroidism, consider measuring TSH, FT4, and FT3 every six weeks until TSH is within reference range, then TSH (with cascading) every three months until antithyroid drugs are stopped.

"For adults who have stopped antithyroid drugs, consider measuring: TSH (with cascading) within 8 weeks of stopping the drug, then TSH (with cascading) every 3 months for a year, then TSH (with cascading) once a year."

"For children and young people who have stopped antithyroid drugs, consider measuring: TSH, FT4 and FT3 within 8 weeks of stopping the drug, then TSH, FT4 and FT3 every 3 months for the first year, then TSH (with cascading) every 6 months for the second year, then TSH (with cascading) once a year."

"Consider measuring TSH every 6 months for adults with untreated subclinical hyperthyroidism. If the TSH level is outside the reference range, consider measuring FT4 and FT3 in the same sample."

"Consider measuring TSH, FT4 and FT3 every 3 months for children and young people with untreated subclinical hyperthyroidism."

"Consider stopping TSH measurement for adults, children and young people with untreated subclinical hyperthyroidism if the TSH level stabilizes (2 similar measurements within the reference range 3 to 6 months apart)" (NICE, 2023).

Society for Maternal-Fetal Medicine (SMFM)

The SMFM recommends against screening asymptomatic pregnant individuals for subclinical hypothyroidism (Society for Maternal-Fetal Medicine, 2022).

Mitochondrial Medicine Society (MMS)

In 2017, the MMS created a working group to provide consensus-based recommendations for optimal management and care for patients with primary mitochondrial disease. From the guidelines, "initial triage stratification of critically ill mitochondrial patients should include a systemic assessment of all body systems since the disease is multisystemic and patients may develop new organ system involvement during an acute decompensation" and thyroid dysfunction can occur in patients with mitochondrial disease, as "both hypothyroidism and, to a far lesser extent, hyperthyroidism have been reported in patients with primary mitochondrial diseases." In addition to routine intensive-care management that might be undertaken for a critically ill patient, they recommend that "thyroid and adrenal function should be assessed in patients at times of critical illness and reassessed during a prolonged intensive care unit stay. Hypo- and hyperglycemia can occur and regular blood glucose monitoring is needed." They also state that "an annual hemoglobin A1c (HgbA1c), thyroid-stimulating hormone, free thyroxine level (FT4), vitamin D, and screening for hypoparathyroidism (serum calcium, magnesium, phosphate, parathyroid hormone, vitamin D (25-OHD and 1,25-OHD); urine: creatinine, calcium, and phosphate) can be considered in individuals with mitochondrial diseases. In those with mtDNA deletions, which are more strongly associated with secondary endocrinopathies, annual screening is recommended" (Parikh et al., 2017).

Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website:https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
80438	Thyrotropin releasing hormone (TRH) stimulation panel; 1 hour This panel must include the following: Thyroid stimulating hormone (TSH) (84443 x 3)
80439	Thyrotropin releasing hormone (TRH) stimulation panel; 2 hour This panel must include the following: Thyroid stimulating hormone (TSH) (84443 x 4)
83519	Immunoassay for analyte other than infectious agent antibody or infectious agent antigen; quantitative, by radioimmunoassay (eg, RIA)

CPT	Code Description
83520	Immunoassay for analyte other than infectious agent antibody or infectious agent antigen; quantitative, not otherwise specified
84432	Thyroglobulin
84436	Thyroxine; total
84439	Thyroxine; free
84442	Thyroxine binding globulin (TBG)
84443	Thyroid stimulating hormone (TSH)
84445	Thyroid stimulating immune globulins (TSI)
84479	Thyroid hormone (T3 or T4) uptake or thyroid hormone binding ratio (THBR)
84480	Triiodothyronine T3; total (TT-3)
84481	Triiodothyronine T3; free
84482	Triiodothyronine T3; reverse
86376	Microsomal antibodies (eg, thyroid or liver-kidney), each
86800	Thyroglobulin antibody

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

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Revision History

Revision Date	Summary of Changes
09/04/2024	Reviewed and Updated: Updated background, guidelines, and evidence-based scientific references. Literature review necessitated the following changes in coverage criteria: CC1 edited to address appropriate type of thyroid function testing for all subcriteria (previously only broken down in CC1a and b). Central hypothyroidism and secondary hypothyroidism are the same, for clarity, wrapped former CC1h into CC1a, added appropriate fT4 monitoring for those diagnosed with secondary hypothyroidism. New CC1.a.v. now reads "v) For individuals being treated for secondary hypothyroidism, monitoring with fT4 testing every 6 weeks upon dosage change and annually in stable individuals." Former CC1.c.iii. is now CC1.c. Edited for clarity, added that TSH is the appropriate screening test. Now reads: "c) For asymptomatic individuals who have been prescribed drugs that can interfere with thyroid function and thus who are at an increased risk for thyroid disease, TSH testing at the following intervals: i) Annually. ii) When dosage or medication changes. iii) If symptoms consistent with thyroid dysfunction develop." TSH is the appropriate marker for CC1.d. New CC1.e. to address all the reasons (former CCs 1.c.i., 1.c.ii., CC1.e., CC1.f., CC1.j, CC1.k) for one time TSH screening: "e) One-time TSH screening: i) For asymptomatic individuals at high risk for thyroid disease due to: (a) Personal or family history of thyroid dysfunction. (b) Personal or family history of type 1 diabetes or other autoimmune disease. ii) For individuals with disease or neoplasm of the thyroid or other endocrine glands. iii) For individuals with chronic or acute urticaria. iv) For pediatric individuals diagnosed with short stature. v) For pediatric individuals with a clinical finding of failure-to-thrive." Formerly CC1.g., now CC1.f., added TSH with reflex fT4 and fT3 when initial result is abnormal, as appropriate marker testing

	New CC1.g., "g) For individuals with hypothalamic-pituitary disease, monitoring of TSH and fT4: i) Biannually for individuals less than 18 years of age. ii) Annually for individuals 18 years of age or older." Former CC1.i., now CC1.h., edited for clarity and consistency. Added CPT code 83520

Urinary Tumor Markers for Bladder Cancer

Policy Number: AHS – G2125 – Urinary Tumor Markers for Bladder Cancer	Prior Policy Name and Number, as applicable:
Initial Presentation Date: 09/18/2015 Revision Date: 03/06/2024	

Policy Description

Bladder cancer is defined as a malignancy that develops from the tissues of the bladder. It is the most common cancer of the urinary system. The cancer typically arises from the urothelium, although it may originate in other locations such as the ureter or urethra (Lerner, 2023).

Tumor biomarkers are proteins detected in the blood, urine, or other body fluids that are produced by the tumor itself or in response to it. Urinary tumor markers may be used to help detect, diagnose, and manage some types of cancer including bladder cancer (Hottinger & Hormigo, 2011).

Related Policies

Policy Number	Policy Title
AHS-G2054	Liquid Biopsy
AHS-G2124	Serum Tumor Markers for Malignancies

Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) Urinary biomarkers (bladder tumor antigen (BTA) test, nuclear matrix protein (NMP22) test, or fluorescence in situ hybridization (FISH) UroVysion Bladder Cancer test) **MEET COVERAGE CRITERIA** in **any** of the following situations:

   a) As an adjunct in the diagnostic exclusion of bladder cancer for individuals who have an atypical or equivocal cytology

   b) As an adjunct in the monitoring of high-risk, non-muscle invasive bladder cancer

2) As an adjunct to cystoscopy or cytology in the monitoring of individuals with bladder cancer, the use of fluorescence immunocytology (ImmunoCyt/uCyt) **MEETS COVERAGE CRITERIA**.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

3) For the evaluation of hematuria, to screen for bladder cancer in asymptomatic individuals, to diagnose bladder cancer in symptomatic individuals, or for any other indication not discussed above, the following tests **DO NOT MEET COVERAGE CRITERIA:**

   a) Urinary biomarkers (bladder tumor antigen (BTA) test, nuclear matrix protein (NMP22) test, or fluorescence in situ hybridization (FISH) UroVysion Bladder Cancer test).

   b) Fluorescence immunocytology (ImmunoCyt/uCyt).

4) Any other urinary tumor markers for bladder cancer not mentioned above **DO NOT MEET COVERAGE CRITERIA**.

Table of Terminology

Term	Definition
AACC	American Association for Clinical Chemistry
ACS	American Cancer Society
AHRQ	Agency for Healthcare Research and Quality
AMH	Asymptomatic microhematuria
ASCO	American Society of Clinical Oncology
ASTRO	American Society for Radiation Oncology
AUA	American Urological Association
AUC	Area under the curve
BC	Bladder cancer
BCG	Bacillus 2 urvivin-guerin
BLCA-1	Bacillus collagen-like protein of anthracis
BLCA-4	Bacillus collagen-like protein of anthracis
BTA	Bladder tumor antigen
CDC	Centers For Disease Control and Prevention
CFHrp	Complement factor h-related protein
CIS	Carcinoma in situ

CK	Cytokeratins
CLIA '88	Clinical Laboratory Improvement Amendments of 1988
CMS	Centers for Medicare and Medicaid Services
CXCR2	C-X-C motif chemokine receptor 2
DNA	Deoxyribonucleic acid
EAU	European Association of Urology
EIA	Enzyme immunoassay
FDA	United States Food and Drug Administration
FISH	Fluorescence in situ hybridization
hCFHrp	Complement factor h-related protein
HTA	Health technology assessment
ICUD-SIU	International Consultation on Urological Diseases & Société Internationale d'Urologie
LDTs	Laboratory-developed tests
MH	Microhematuria
MRI	Magnetic resonance imaging
NACB	National Academy of Clinical Biochemistry Laboratory Medicine
NCCN	National Comprehensive Cancer Network
NCI	National Cancer Institute
NED	Non-evidence of disease
NID2	Nidogen 2
NMIBC	Non-muscle invasive bladder cancer
NMP22	Nuclear matrix protein 22
NMP52	Nuclear matrix protein 52
PCR	Polymerase chain reaction
RCTs	Randomized controlled trials
SUFU	Society of Urodynamics, Female Pelvic Medicine & Urogenital Reconstruction
SUO	Society of Urologic Oncology
TWIST1	Twist-related protein 1
uCyt+	*ImmunoCyt* test
USPSTF	U.S. Preventive Services Task Force
UT	Urine derived tumor
utDNA	Urine derived tumor deoxyribonucleic acid

Scientific Background

Each year in the United States, the American Cancer Society estimates there are about 82,290 new cases of bladder cancer and about 16,710 deaths from bladder cancer (ACS, 2023). Bladder cancer is the sixth most common cancer in the United States, affects men four times more frequently than women, and is typically diagnosed in individuals above the age of 40, with 73 the median age at diagnosis (DeGeorge

et al., 2017; NCCN, 2023a). Bladder cancer risk factors include smoking, a family history of the disease, pelvic radiation, obesity, diabetes, and chronic infection of the urinary tract.

Bladder cancer commonly presents as painless hematuria (blood in urine) and may be gross (visible) or microscopic. Gross hematuria tends to increase the likelihood of bladder cancer, but hematuria as a whole may be transient or due to non-cancer related causes (Perazalla, 2021). Other common symptoms of bladder cancer include pain or irritative and obstructive voiding symptoms such as urge incontinence, dysuria, straining, or nocturia. These symptoms are often mistaken for another condition such as kidney stones, can be temporary, and are not necessarily specific for bladder cancer (Lotan & Choueiri, 2022). In fact, hematuria is the most common symptom of bladder cancer, but a study reported a 13% prevalence rate of bladder cancer out of 6728 patients with hematuria (DeGeorge et al., 2017; Sutton et al., 2018). Approximately 70%-75% of patients present with superficial tumors (50 – 70% of which can recur but are usually not life threatening), and 25%-30% present as invasive tumors with a high risk of metastasis (Chou & Dana, 2010; Kaufman et al., 2009).

Cystoscopy (white light) is the gold standard for a diagnosis of bladder cancer. This procedure involves a bladder examination and urine sample for cytology. Any lesions are observed and recorded. Cystoscopy does not detect all malignancies or visualize the upper urinary tract. Furthermore, although cystoscopy is minimally invasive, it may be uncomfortable and promote anxiety, which can lead to suboptimal compliance with management recommendations. Fluorescent cystoscopy is somewhat more efficient at detecting tumors than white light cystoscopy; although, it comes with its own set of issues such as higher false-positive rates and costs (Lotan & Choueiri, 2022; Mitra et al., 2023). Cytology, or the analysis of cells in urine, is often completed in addition to cystoscopy analysis.

Although cystoscopy has long been the gold standard for a diagnosis of bladder cancer, its high cost and unpleasant burden has led to the search for a non-invasive test that can match the high specificities and sensitivities set by cystoscopy. Urinary biomarkers including "Cell-free proteins and peptides, exosomes, cell-free DNA, methylated DNA and DNA mutations, circulating tumor cells, miRNA, lncRNA, rtRNA and mRNAs" have now been identified for bladder cancer diagnostic purposes (Lopez-Beltran et al., 2019). Urine is exposed to urothelial tissue in many different locations, and therefore has the potential to contain several biomarkers associated with cancer. Validation of these biomarkers could lessen the use of cystoscopy as well as increase the overall sensitivity for bladder cancer identification (D'Costa et al., 2016). However, because of the lower disease prevalence in a screening population, even in those at increased risk, the use of biomarkers for screening is not cost effective or recommended (Lotan et al., 2009). Despite the promise of urine biomarkers, cystoscopy remains the procedure of choice both for initial diagnosis and for surveillance in previously treated patients.

Epigenetic changes may also play an important role in bladder cancer tumorigenesis. These changes are becoming more prevalent as identification rates increase due to improvements in high-throughput DNA sequencing technologies. Epigenetic changes can "regulate [the] gene expression outcome without changing the underlying DNA sequence" with alterations based on DNA methylation, nucleosome positioning, microRNA regulation and histone medications. All these epigenetic-based changes are distorted in each human cancer type. "A substantial portion (76%) of all primary bladder tumors displays mutations in at least one chromatin regulatory gene. These mutations cause epigenetic dysregulation in bladder cancers" (Li et al., 2016).

Numerous other urinary biomarkers have been proposed as contributors to management of bladder cancer.

Other nuclear matrix proteins aside from NMP22 have been investigated. NMP52, BLCA-4, and BLCA-1 have all been studied as potential markers. Initial data for these markers appears promising, but most likely requires further evaluation (Mitra et al., 2023).

Cytokeratins, protein components of the cell structure, have also been identified as possible markers. Cytokeratins ("CK"), -8, -18, -19, and -20 have been considered for use in bladder cancer evaluation. However, further data is needed (Mitra et al., 2023).

Other markers that have been considered as potential indicators of bladder cancer include the following:

Telomerase is an enzyme that adds telomeres to the ends of chromosomes. This enzyme is only expressed in proliferating cells such as cancer cells, thereby lending credence to its use as a cancer marker. Despite its high sensitivity, its clinical application is limited, as the current assay used to detect telomerase is "significantly" affected by sample collection and processing (Mitra et al., 2023).

Hyaluronic acid is a polysaccharide that promotes tumor progression and metastasis. It is cleaved by *hyaluronidase*, which creates smaller fragments of the polysaccharide that further promote tumor angiogenesis. This pair of markers has been found to detect low-grade and low-stage disease with higher sensitivities than other markers, but requires further data for evaluation (Mitra et al., 2023).

Fibrin degradation products may also be useful in detection of cancer. High levels of vascular endothelial growth factor can increase the permeability of surrounding cellular structures, which cause serum proteins to "leak." These proteins are eventually degraded to fibrin, and then to fibrin degradation products (Mitra et al., 2023).

Survivin is an apoptosis inhibitor. Survivin is frequently elevated in cancers, but virtually undetectable in normal tissues. However, no commercial assays for Survivin exist as of time of writing (Mitra et al., 2023).

Finally, *miRNA* markers have been considered for use in bladder cancer management. These markers are small sequences of non-coding RNA that contribute to gene expression regulation. MiRNAs-126, -200c, -143, and -222 have all been considered to have "promising" results (Mitra et al., 2023).

Proprietary Testing

The two most studied urinary biomarkers are bladder tumor antigen (BTA) and nuclear matrix protein 22 (NMP22). The BTA test is designed to detect complement factor H-related protein (hCFHrp) which is elevated in cancer cells. This test is available in both a quantitative and qualitive version, and its manufacturer-recommended cut-off is 14U/Ml (Mahnert et al., 1999; Mitra et al., 2023). The BTA stat® test and the BTA TRAK® test are available from Polymedco and measure qualitative and quantitative detection of bladder tumor-associated antigen, respectively. Similarly, the NMP22 test is designed to detect a protein that is more highly available in cancer cells than normal cells. In this case, cancer cells release more NMP22 into the urine following apoptosis than normal cells do. The NMP22 tests are also available in a quantitative and qualitative version, and its FDA-approved cut-off is 10U/Ml (Grossman et al., 2005; Mitra et al., 2023; Zuiverloon et al., 2017). A number of proprietary tests exist revolving around one of these two biomarkers; these tests include Abbott's "Alere NMP22 BladderCheck" and Quest's Bladder Tumor Antigen DetectR (Abbott, 2023; Quest, 2020).

The FDA has approved two additional tests for urinary biomarkers. One is *UroVysion*, which is designed to detect chromosomal alterations that are distinctive of bladder cancer. This test is a fluorescent in situ hybridization (FISH) assay that uses DNA probes to detect alterations (such as aneuploidies) on

chromosomes 3, 7, and 17 or loss of the 9p21 locus. The second test is known as *ImmunoCyt* (or uCyt+) that uses a similar fluorescent technique to detect certain glycoproteins that are expressed solely on cancerous cells (Mitra et al., 2023).

Recently, Pangea Laboratory has created a laboratory developed test termed Bladder *CARE*™ which measures the methylation status of specific DNA biomarkers in urine for the detection of bladder cancer via an at-home collection kit. This non-invasive test has not been approved by the FDA, is purported to be more cost-effective, and uses an epigenetic-based detection approach. Specifically, the methylation of bladder cancer DNA biomarkers are measured (Pangea, 2020). As little as 5 ng of urine DNA from a 100 Ml urine sample is required, and it has a limit detection of 0.1% leading to the identification of a single cancerous cell in a sample of 1,000 normal cells (Pangea, 2020). The authors claim that Bladder *CARE*™ has a sensitivity of 94% and specificity of 86%, allowing for the identification of 88% of low-grade bladder cancer cases; these results are based on a study completed by Pangea Laboratory and Zymo Research which analyzes urine samples from 182 patients (97 with bladder cancer and 85 healthy controls) (Pangea, 2019).

Another test, termed the Bladder EpiCheck test, has been developed by the Israeli company Nucleix. This non-invasive epigenetic urine test helps to detect bladder cancer with a panel of 15 DNA methylation biomarkers. Nucleix reports a sensitivity of 92%, a specificity of 88% and a negative predictive value of 99% for the Bladder EpiCheck test; these results are based on a multi-center clinical study with 353 bladder cancer patients (Nucleix, 2015). Similar results have been reported by D'Andrea et al. (2019). However, this test is not available in the United States (Nucleix, 2015).

Another test, termed "UBC® Rapid" has been developed by the Swedish company ODL Biotech. This point-of-care test measures soluble fragments of cytokeratins 8 and 18 in urine samples. The test can produce results within 10 minutes and may be tested with hematuria-containing samples. UBC® Rapid is the only quantitative point of care test platform for urine-based detection of bladder cancer. (AroCell, 2023). Ecke et al. (2018) performed a validation of this test, which encompassed 242 patients with bladder cancer (134 non-muscle-invasive low-grade tumors, 48 non-muscle-invasive high-grade tumors, 60 muscle-invasive high-grade tumors), 62 patients with non-evidence of disease [NED], and 226 healthy controls. The authors found a sensitivity of 38.8% for non-muscle-invasive low-grade bladder cancer, 75% for non-muscle-invasive high-grade bladder cancer and 68.3% for muscle-invasive high-grade bladder cancer. Specificity over the entire cohort was 93.8% (Ecke et al., 2018).

The URO17 assay by Protean Biodiagnostics, an immunohistochemistry-based test that detects the presence of the oncoprotein keratin 17 in bladder cancer and urogenital cancer. Unlike other urine-based test URO17 can detect patients with visible or invisible hematuria, which allows for early diagnosis. URO17 can also detect recurrent bladder cancer in patients under surveillance for relapse (NICE, 2023). The test has 100% sensitivity and 96% specificity for detecting bladder cancer from urine samples (Protean Biodiagnostics, 2021).

Nonagen Bioscience released Oncuria, an in-vitro multiplex immunoassay, which detects protein biomarkers associated with bladder cancer in the urine. This non-invasive test detects ten proteins from a single urine sample in patients with hematuria with suspicion of bladder cancer. Biomarker levels are combined in a weighted algorithm to aid in the prediction of responding to Bacillus Calmette-Guerin (BCG) therapy in patients with intermediate to high-risk, early-stage bladder cancer (Nonagen Bioscience, 2022).

The Xpert® Bladder Cancer Monitor can be used as a diagnostic in a population of patients with a history of non-muscle invasive bladder cancer (NMIBC). The test was designed for use in follow-up of patients undergoing routine surveillance. Pichler et al. (2018) enrolled 140 patients with a history of NMIBC and the patients underwent urine cytology using the Paris classification system. Urinary specimens were also analyzed with PCR using the Xpert® BC monitor, which measures five target mRNAs (ABL1, CRH, IGF2, UPK1B, and ANXA10). The overall sensitivity of the Xpert® BC Monitor was 0.84 with an NPV of 0.93. The authors write that this was "significantly superior" to the sensitivity of bladder washing cytology (0.33 and 0.76; $P < 0.001$). Another subgroup analysis confirmed the sensitivity as compared to barbotage cytology (Pichler et al., 2018).

D'Elia et al. (2021) also performed a study tracking follow-up and diagnostic utility of the Xpert® BC for patients with a history of NMIBC. This prospective study was done using 1015 samples from a group of 416 patients. Patients had a urinary cytology, the Xpert® Bladder Cancer monitor test, and cystoscopy. If the cystoscopy was positive, a transurethral resection of the bladder was completed. The Xpert® BC test identified 168 recurrent tumors: 126 were low-grade and 42 were high-grade; the overall sensitivity was 17.9% for cytology, 52.4% for the Xpert® BC test and 54.2% for the two tests combined. Overall specificity was 98.5% for cytology, 78.4% for the Xpert® BC test, and 78.2% for the two tests combined (D'Elia et al., 2021).

Analytical Validity

Piao et al. (2019) have developed a way to differentiate patients with bladder cancer from patients with a nonmalignant hematuria without bladder cancer by measuring urinary cell-free microRNA expression. This study shows that the non-invasive measurement of urinary microRNA-6124 and microRNA-4511 can be used as a diagnostic tool with a sensitivity of >90% (Piao et al., 2019). This testing method will help to reduce the number of unnecessary cystoscopies in patients with hematuria that are being evaluated for bladder cancer.

The performance of an epigenetic-based bladder cancer detection tool has been evaluated by Fantony et al. (2017); the urine-based TWIST1/NID2 methylation assay has been analyzed for the detection of urothelial carcinoma via the addition of urine cytology. This multi-institutional study analyzed data from 172 patients. The authors note that "The AUC [area under the curve] for cytology alone with equivocal cytologies positive was 0.704 and improved to 0.773 with the addition of the DNA methylation assay ($p < 0.001$)" (Fantony et al., 2017). The authors conclude by stating that this TWIST1/NID2 methylation assay is a sensitive diagnostic tool that adds value to urine cytology for the detection of urothelial carcinoma, which is the most common type of bladder cancer.

Soubra and Risk (2015) found the sensitivity of fluorescent cystoscopy to be 0.92 and the sensitivity of white light cystoscopy to be 0.71; the specificity of fluorescent cystoscopy was lower at 0.57, and the specificity of white light cystoscopy was identified at 0.72. Furthermore, fluorescent cystoscopy's sensitivity for carcinoma *in situ* (which is difficult to visualize) was measured at 0.924, while white light cystoscopy's sensitivity for carcinoma *in situ* was much lower at 0.605, but these differences tended to decrease on higher grade lesions (Soubra & Risk, 2015). Cytology is also a common analytic technique in addition to cystoscopy. Its overall sensitivity is low at 0.34 and its sensitivity for grade 1 and 2 tumors is even lower at 0.12 and 0.26, respectively (Lotan & Roehrborn, 2003).

Breen et al. (2015) compared the sensitivity and specificity values of four diagnostic tests (cytology, NMP22, UroVysion, and CxBladder); CxBladder was found to have the highest sensitivity at 74% and cytology was identified with the highest specificity at 95%. The authors report comparable sensitivity

values for cytology, NMP22, and UroVysion at 46%, 45.9% and 47.7% respectively (Breen et al., 2015). It is important to note that even though CxBladder is reported to have the highest sensitivity, the specificity (81.7%) is the lowest; the other tests were reported to have superior specificities with NMP22 at 88%, and UroVysion at 87.7% (Breen et al., 2015).

Sathianathen et al. (2018) published a study focusing on biomarkers in patients presenting with hematuria. This study encompassed BTA, NMP22, FISH, and uCyt+, as well as a fifth biomarker known as AssureMDx. Sensitivities ranged from 0.67 (BTA) to 0.95 (AssureMDx, second highest was uCyt+ at 0.83) while specificities ranged from 0.68 (BTA) to 0.93 (quantitative NMP22). However, this data is consistent with the previously published meta-analysis that covered all settings, not just hematuria (Chou et al., 2015). Cytology was also found to have superior specificity to all studied biomarkers; although, biomarkers tended to have better sensitivity. The authors concluded that, due to the high heterogeneity and small sample size, more studies were needed to validate biomarkers to replace diagnostic evaluation of hematuria (Sathianathen et al., 2018).

Although many studies emphasize the high validity of biomarkers such as NMP22 and BTA, these studies often have a large proportion of high-grade tumors which inflate the specificity and sensitivity; hence, the problem of identifying low-grade cancers remains. There may be changes at the genetic level in a low-grade cancer, but the proteins tested in the urine may still be relatively normal (D'Costa et al., 2016). Another issue is the conflicting results for the validity of the biomarkers. For example, the sensitivity of the quantitative NMP22 test has been found to range from as low as 0.26 to 1.00 with its specificity ranging from 0.49 to 0.98. Similarly, the *BTA STAT* test's sensitivity and specificity have been found to range from 0.29 to 0.91 and from 0.54 to 0.86 respectively (Zuiverloon et al., 2017). For comparison, a study found the sensitivity and specificity of flexible cystoscopy (out of 778 hematuria patients) to be 0.98 and 0.938, respectively (Sutton et al., 2018).

Dudley et al. (2019) have developed a novel high-throughput sequencing method that uses urine derived tumor DNA (utDNA) known as utDNA CAPP-Seq (Ucapp-Seq) to detect bladder cancer. This technique was used to analyze samples from 118 patients with early-stage bladder cancer and 67 healthy adults. "We detected utDNA pretreatment in 93% of cases using a tumor mutation-informed approach and in 84% when blinded to tumor mutation status, with 96% to 100% specificity" (Dudley et al., 2019). These results show that utDNA can be used to diagnose early-stage bladder cancer with high sensitivity and specificity.

Hirasawa et al. (2021) studied the diagnostic performance of Oncuria™, a multiplex immunoassay urinalysis test for bladder cancer. Urine samples from 362 subjects with suspicion of bladder cancer were measured using Oncuria™ for ten biomarkers (A1AT, APOE, ANG, CA9, IL8, MMP9, MMP10, PAI1, SDC1 and VEGFA). Results of the test were confirmed by cystoscopy and tissue biopsy. "The Oncuria™ test achieved a strong overall diagnostic performance, achieving an overall AUC of 0.95, sensitivity and specificity values of 93% and 93%, respectively, and a negative predictive value (NPV) and positive predictive value (PPV) of 99% and 65%, respectively. The Oncuria™ test shows promise for clinical application in the non-invasive diagnosis and surveillance bladder cancer, and potentially for screening at-risk, asymptomatic individuals" (Hirasawa et al., 2021).

Clinical Utility and Validity

A meta-analysis of 57 studies detailed the accuracy of several biomarkers for the diagnosis and surveillance of bladder cancer. These included the six FDA-approved tests (quantitative and qualitive NMP22, quantitative and qualitative BTA, FISH, and uCyt+) as well as a laboratory developed test that

does not require FDA approval termed CxBladder. Sensitivities ranged from 0.57 (qualitative NMP22) to 0.82 (CxBladder); however, the CxBladder cohort was only comprised of one study. The specificities ranged from 0.74 (quantitative BTA) to 0.88 (qualitative NMP22). Sensitivity increased as a tumor progressed (higher grade or stage) with low accuracy for lower stage or grade tumors. A cytologic evaluation performed with a biomarker assessment increased sensitivity as well but missed about 10% of cases. Ultimately, the authors concluded that urinary biomarkers reported many false-positive results and failed to identify a large percentage of patients with bladder cancer (Chou et al., 2015). The authors also noted that this was the first study which focused on the measurement of clinical outcomes based on urinary biomarkers.

The ideal marker will be "easier, better, faster, and cheaper" (Schmitz-Dräger et al., 2015). Overall, although there have been numerous promising studies for the clinical utility of these urinary biomarkers, the biomarkers do not yet measure up to the standards set by cystoscopy as the primary method of diagnosis. Most of the biomarkers are yet to be well-validated and the ones that are, such as NMP22 and BTA, fall short of cystoscopy's standards (D'Costa et al., 2016). Furthermore, because of the lower disease prevalence in a screening population, even in those at increased risk, the use of biomarkers for screening is not cost effective or recommended (Lotan et al., 2009). Although the cost of tests is non-clinical, it is still a crucial issue; the *BTA* and *NMP22* tests are relatively inexpensive at $25 but ImmunoCyt costs around $80 and the CxBladder and UroVysion cost $325 and $800, respectively (Zuiverloon et al., 2017). For comparison, a cystoscopy cost around $210 in 2016, and a cystoscopy with a biopsy cost about $370 (Halpern et al., 2017). These biomarkers to date have not been highly recommended within any clinical guidelines. Therefore, the authors concluded that biomarkers have not had significant effect on clinical decision-making (Schmitz-Dräger et al., 2015).

An in-depth health technology assessment (HTA) of Cxbladder test was performed by Landaas et al. (2020) integrating clinical data and real-world usage scenarios to highlight the test's sensitivity and specificity. Data from a vendor-funded study showed sensitivity of 91% and specificity of 60% for Cxbladder; another study indicated a sensitivity of 0.82 and specificity of .85. The authors also noted an Agency for Healthcare Research and Quality (AHRQ)-funded systematic review by Chou et al. (2015) highlighting the high false-positive rate and poor accuracy of Cxbladder for low-stage and low-grade tumors. The AHRQ concluded that urinary biomarkers like Cxbladder would miss a substantial portion of bladder cancer cases and tests were subject to false positive results (Chou et al., 2015).

A follow-up pilot study by Landaas et al. (2020) was initiated at UW Medicine to analyze the best use-case scenario for Cxbladder. The pilot study involved patients with a history of urothelial carcinoma, comparing those tests with Cxbladder (group 1) to a control group (group 2). Group 1 patients underwent various follow-up tests including urine cytologies, cystoscopies, and biopsies, with recurrence detected in two out of six patients within the study period. Group 2, without Cxbladder testing, had three out of six patients with detected recurrence. The study essentially found no significant differences in follow-up tests between the two groups. These findings underscore the complexities of adopting new molecular diagnostic tests like Cxbladder on a system-wide basis. However, the study did find that Cxbladder testing *was* beneficial for a specific patient profile: those with normal cystoscopy results and atypical cytology. In such cases. Cxbladder testing led to fewer follow-up procedures (cystoscopies, cytologies, and biopsies) while still detecting a similar proportion of bladder cancer recurrences as standard procedures within the year. In conclusion, Cxbladder appears most suitable for those undergoing surveillance for bladder cancer recurrence, particularly those with normal cystoscopy and atypical cytology (Landaas et al., 2020).

The majority of studies performed on these biomarkers did not focus on their ability to predict the course of cancer (D'Costa et al., 2016) but some biomarkers may play a role in the diagnosis or surveillance of bladder cancer in the future (Schmitz-Dräger et al., 2015). Even this may be a difficult barrier to cross; Meleth et al. (2014) prepared an assessment for the Agency for Healthcare Research and Quality that stated "although UroVysion is marketed as a diagnostic rather than a prognostic test, limited evidence from two small studies (total n=168) supported associations between test result and prognosis for risk of recurrence" (Meleth et al., 2014). The authors went on to note that no studies that established clinical utility were found.

D'Andrea et al. (2019) analyzed 357 urine samples from patients at five different centers under surveillance for non-muscle-invasive bladder cancer to investigate the clinical utility of the Bladder EpiCheck™ non-invasive urine test. A specificity of 88% was identified with this test, a negative predictive value of 94.4% for the detection of any cancer, and a negative predictive value of 99.3% for the detection of high grade cancer; the use of the Bladder EpiCheck™ test helped to improve the cancer recurrence predictive value by a difference of 16-22% (D'Andrea et al., 2019). This high-performing diagnostic test may help in the surveillance of non-muscle-invasive bladder cancer.

Tan et al. (2018) completed a systematic review to identify the diagnostic sensitivity and specificity of urinary biomarkers for the diagnosis of bladder cancer. The authors report that multi-target biomarker panels were more accurate than single biomarker targets, and that both the sensitivity and specificity of urinary biomarkers were higher in primary diagnostic scenarios compared to patients under surveillance (Tan et al., 2018). The authors note that "few biomarkers achieve a high sensitivity and negative predictive value," with single biomarkers reporting a sensitivity of 2-94% and specificity of 46-100%, and multi-target biomarkers reporting a sensitivity of 24-100% and specificity of 48-100% (Tan et al., 2018).

Mossanen et al. (2019) performed a cost analysis to characterize the costs of managing non-muscle-invasive bladder cancer (NMIBC). The authors created a Markov model with four health states: no evidence of disease, recurrence, progression and cystectomy, and death. Patients were stratified into three risk categories of low, intermediate, and high. The authors found that "cumulative costs of care over a 5-year period were $52,125 for low-risk, $146,250 for intermediate-risk, and $366,143 for high-risk NMIBC." The authors identified that the primary driver of cost was "progression to muscle-invasive disease requiring definitive therapy", which was found to contribute 81% and 92% to overall cost for intermediate and high-risk disease, respectively. Progression of disease was found to contribute 71% to overall cost for low-risk disease. The authors concluded that although protracted surveillance cystoscopy does contribute to management cost, progression of disease was the dominant factor in increasing cost of care (Mossanen et al., 2019).

Vasdev et al. (2021) studied the role of URO17™ biomarker in the diagnosis of bladder or urothelial cancer in new hematuria patients. Urine samples from 71 subjects were stained using the URO17™ immunobiomarker and results were compared to the biopsy and histology. URO17™ was shown to have an overall sensitivity of 100%, specificity of 92.6%, positive predictive value of 0.957, and negative predictive value of 1. URO17™ investigation was positive in every case of urothelial malignancy. According to the authors, URO17™ test can help improve "diagnostic capabilities in primary care, reduce the number of referrals to Urology department, and reduce the number of unnecessary invasive procedures for new patients with a suspected urinary bladder cancer" (Vasdev et al., 2021).

Guidelines and Recommendations

National Comprehensive Cancer Network (NCCN)

The NCCN has stated that "Urine molecular tests for urothelial tumor markers are now available. Many of these tests have a better sensitivity for detecting bladder cancer than urinary cytology, but specificity is lower. Considering this, evaluation of urinary urothelial tumor markers may be considered during surveillance of high-risk non-muscle-invasive bladder cancer. However, it remains unclear whether these tests offer additional information that is useful for detection and management of non-muscle-invasive bladder tumors. Therefore, the panel considers this to be a category 2B recommendation" (NCCN, 2023b).

American Urological Association (AUA)

The AUA's guidelines on the diagnosis, evaluation and follow-up of asymptomatic microhematuria (AMH) in adults do not recommend use of urine markers (NMP22, BTA-stat, UroVysion) as part of routine evaluation (Davis et al., 2012).

The AUA and Society of Urodynamics, Female Pelvic Medicine & Urogenital Reconstruction (SUFU) published a guideline on microhematuria in 2020. In it, they remark that "Clinicians should not use urine cytology or urine-based tumor markers in the initial evaluation of patients with microhematuria", stating that "insufficient evidence exists that routine use would improve detection of bladder cancer." However, the guideline states that "Clinicians may obtain urine cytology for patients with persistent microhematuria after a negative workup who have irritative voiding symptoms or risk factors for carcinoma in situ." Overall, the guideline states that "the panel does not recommend using urine cytology or urine-based tumor markers in the initial evaluation of MH [microhematuria] because, to date, markers have not demonstrated incrementally additive information to cystoscopy in the MH population, not have they been found to be of sufficient predictive value to obviate cystoscopy" (Barocas et al., 2020).

The AUA and Society of Urologic Oncology (SUO) joint guidelines on Diagnosis and Treatment of Non-Muscle Invasive Bladder Cancer (NMIBC) do not recommend using urinary biomarkers to replace cystoscopy when monitoring NMIBC (grade B), although a clinician can use biomarkers to evaluate a patient's response to Bacillus Calmette-Guerin (BCG) therapy or a separate cytology such as FISH or ImmunoCyt. However, a urinary biomarker should not be used for monitoring a patient with a normal cystoscopy and a history of low-risk cancer (Chang et al., 2020). This 2016 guideline was amended in 2020, but no relevant changes were identified.

The 2021 American Urologic Association (AUA) annual meeting included a guideline amendment update for non-muscle invasive bladder cancer (NMIBC) and muscle-invasive bladder cancer (MIBC) to the 2020 guidelines. According to the update, a clinical should not use urinary biomarkers in place of cystoscopy. "In a patient with a history of low-risk cancer and a normal cystoscopy, a clinician should not routinely use a urinary biomarker or cytology during surveillance. In a patient with NMIBC, a clinician may use biomarkers to assess response to intravesical BCG (UroVysion® FISH) and adjudicate equivocal cytology (UroVysion® FISH and ImmunoCyt™)" (AUA/SUO, 2020). The panel does acknowledge the uptake of Cxbladder in clinical practice; however, there is a lack of high quality evidence in the potential replacement of cystoscopy with Cxbladder (AUA, 2021).

Similarly, the joint guidelines between the AUA, the SUO, the American Society of Clinical Oncology (ASCO), and the American Society for Radiation Oncology (ASTRO) regarding non-metastatic muscle-invasive bladder cancer note that molecular biomarkers may be important for staging cancer and deciding a course of treatment soon. Nevertheless, at this time the biomarkers have not been properly validated (Chang et al., 2017).

U.S. Preventive Services Task Force (USPSTF)

The USPSTF concluded in 2011 that there was insufficient evidence to evaluate screening for bladder cancer in asymptomatic adults, assigning a grade I to this recommendation. Since then, there have been no further guidelines published on this topic by the USPSTF (Moyer, 2011).

In 2021, the USPSTF published the following statement regarding bladder cancer screening in adults: "Literature scans conducted in November 2021 in the MEDLINE and PubMed databases and the Cochrane Library showed a lack of new evidence to support an updated systematic review on the topic at this time (USPSTF, 2021).

3rd International Consultation on Urological Diseases & Société Internationale d'Urologie (ICUD-SIU)

With a level of evidence of 3 and a grade of "B", the ICUD-SIU recommends, "examination of urine cytology must be a part of the expectant management or active surveillance protocol." Concerning the surveillance strategies for NMIBC, "Surveillance strategies following a negative 3 months surveillance cystoscopy should be: (1) for low-risk disease, cystoscopy 6–9 months later and annually thereafter; consider cessation following five recurrence-free years. No upper tract imaging necessary unless hematuria present; (2) for intermediate risk, cystoscopy with cytology every 3–6 months for 2 years; then every 6–12 months during years 3 and 4; then annually for lifetime. Upper tract imaging every 1–2 years; (3) for high risk, cystoscopy with cytology every 3 months for 2 years; then every 6 months during years 3 and 4; then annually for lifetime [Level of evidence: 3; Grade C]" (Monteiro et al., 2018).

National Cancer Institute (NCI)

In the 2022 update to the NCI's *Bladder and Other Urothelial Cancers Screening (PDQ®)—Health Professional Version*, the NCI states that "There is inadequate evidence to determine whether screening for bladder and other urothelial cancers has an impact on mortality… Based on fair evidence, screening for bladder and other urothelial cancers would result in unnecessary diagnostic procedures with attendant morbidity" (NCI, 2022) .

European Association of Urology (EAU)

The EAU has published guidelines on non-muscle-invasive bladder cancer (NIBC).

. In 2022, the EAU concluded that "Cystoscopy is necessary for the diagnosis of bladder cancer" and that "Urinary cytology has high sensitivity in high-grade tumours including carcinoma in situ." The EAU remarks that "There is no known urinary marker specific for the diagnosis of invasive BC [bladder cancer]" (Witjes et al., 2022).

An update to guidelines on non-muscle-invasive bladder cancer (NIBC) was published in 2022. The EAU concluded that urinary molecular marker tests cannot replace cystoscopy in routine practice, "but the knowledge of positive test results (microsatellite analysis) can improve the quality of follow-up cystoscopy." Diagnosis ultimately depends on "cystoscopy examination of the bladder and histological evaluation of sampled tissue" (Babjuk et al., 2022).

An update to the EAU guidelines was published in 2023. In it, the EAU commented on urinary molecular marker tests, "None of these markers have been accepted as routine practice by any clinical guidelines for diagnosis or follow-up." However, they remarked that "promising urinary biomarkers, assessing

multiple targets, have been tested in prospective multicentre studies. Four of the promising and commercially available urine biomarkers, Cxbladder, ADX-Bladder, Xpert Bladder and EpiCheck, although not tested in RCTs, have such high sensitivities and negative predictive values in the referenced studies for high grade disease that these biomarkers may approach the sensitivity of cystoscopy. These 4 tests might be used to replace and/or postpone cystoscopy as they may identify the rare HG recurrences occurring in low/intermediate NMIBC" (EAU, 2023).

Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: http://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

On April 16, 1997, the FDA approved the *Bard BTA stat™ Test*, created by Bard Diagnostic Sciences Inc. From the FDA site: "the BTA stat test is an *in vitro* diagnostic immunoassay indicated for the qualitative detection of bladder tumor associated antigen in urine of persons diagnosed with bladder cancer. This test is indicated for use as an aid in the management of bladder cancer patients in conjunction with cystoscopy."

On April 15, 1998, the FDA approved the *BTA TRAK™ Test*, created by Bard Diagnostic Sciences Inc. From the FDA site: "the BTA TRAK test is an *in vitro* diagnostic immunoassay indicated for the quantitative detection of bladder tumor associated antigen in human urine. This test is indicated for use as an aid in the management of bladder cancer patients in conjunction with cystoscopy."

On July 2, 1996, the FDA approved the *MATRITECH NMP22™ TEST KIT*, created by Alere Scarborough Inc. From the FDA site: "The Matritech NMP22 Test Kit is an enzyme immunoassay (EIA) for the in vitro quantitative determination of nuclear matrix protein NMP22 in stabilized voided urine."

On July 30, 2002, the FDA approved the *NMP22 BladderChek*, created by Matritech Inc. From the FDA site: "The Matritech NMP22 BladderChek Test is indicated for professional and prescription home use as an aid in monitoring bladder cancer patients, in conjunction with standard diagnostic procedures." This assay is qualitative.

On January 24, 2005, the FDA approved the *UROVYSION BLADDER CANCER KIT*. From the FDA site: "The UroVysion Bladder Cancer Kit (UroVysion Kit) is designed to detect aneuploidy for chromosomes 3, 7, 17, and loss of the 9p21 locus via fluorescence in situ hybridization (FISH) in urine specimens from persons with hematuria suspected of having bladder cancer."

On February 23, 2000, the FDA approved the *ImmunoCyt,* created by Diagnocure Inc. From the FDA site: "ImmunoCyt is a qualitative direct immunofluorescence assay intended for use in conjunction with cytology to increase overall sensitivity for the detection of tumor cells exfoliated in the urine of patients previously diagnosed with bladder cancer. ImmunoCyt is indicated for use as an aid in the management of bladder cancer in conjunction with urinary cytology and cystoscopy" (FDA, 2018).

All of the FDA-approved tests apart from ImmunoCyt are approved for both diagnosis and surveillance of bladder cancer whereas ImmunoCyt is only approved for surveillance (Darwiche et al., 2015).

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
86294	Immunoassay for tumor antigen, qualitative or semiquantitative (eg, bladder tumor antigen)
86316	Immunoassay for tumor antigen, other antigen, quantitative (eg, CA 50, 72-4, 549), each
86386	Nuclear Matrix Protein 22 (NMP22), qualitative
88120	Cytopathology, in situ hybridization (eg, FISH), urinary tract specimen with morphometric analysis, 3-5 molecular probes, each specimen; manual
88121	Cytopathology, in situ hybridization (eg, FISH), urinary tract specimen with morphometric analysis, 3-5 molecular probes, each specimen; using computer-assisted technology
88346	Immunofluorescence, per specimen; initial single antibody stain procedure
88350	Immunofluorescence, per specimen; each additional single antibody stain procedure (List separately in addition to code for primary procedure)
0012M	Oncology (urothelial), mRNA, gene expression profiling by real-time quantitative PCR of five genes (MDK, HOXA13, CDC2 [CDK1], IGFBP5, and CXCR2), utilizing urine, algorithm reported as a risk score for having urothelial carcinoma Proprietary test: Cxbladder™ Detect Lab/manufacturer: Pacific Edge Diagnostics USA, Ltd
0013M	Oncology (urothelial), mRNA, gene expression profiling by real-time quantitative PCR of five genes (MDK, HOXA13, CDC2 [CDK1], IGFBP5, and CXCR2), utilizing urine, algorithm reported as a risk score for having recurrent urothelial carcinoma Proprietary test: Cxbladder™ Monitor Lab/manufacturer: Pacific Edge Diagnostics USA, Ltd
0363U	Oncology (urothelial), mRNA, gene-expression profiling by real-time quantitative PCR of 5 genes (MDK, HOXA13, CDC2 [CDK1], IGFBP5, and CXCR2), utilizing urine, algorithm incorporates age, sex, smoking history, and macrohematuria frequency, reported as a risk score for having urothelial carcinoma Proprietary test: Cxbladder™ Triage Lab/Manufacturer: Pacific Edge Diagnostics USA, Ltd
0365U	Oncology (bladder), analysis of 10 protein biomarkers (A1AT, ANG, APOE, CA9, IL8, MMP9, MMP10, PAI1, SDC1 and VEGFA) by immunoassays, urine, algorithm reported as a probability of bladder cancer Proprietary test: Oncuria® Detect Lab/Manufacturer: DiaCarta Clinical Lab
0366U	Oncology (bladder), analysis of 10 protein biomarkers (A1AT, ANG, APOE, CA9, IL8, MMP9, MMP10, PAI1, SDC1 and VEGFA) by immunoassays, urine, algorithm reported as a probability of recurrent bladder cancer

CPT	Code Description
	Proprietary test: Oncuria® Monitor Lab/Manufacturer: DiaCarta Clinical Lab
0367U	Oncology (bladder), analysis of 10 protein biomarkers (A1AT, ANG, APOE, CA9, IL8, MMP9, MMP10, PAI1, SDC1 and VEGFA) by immunoassays, urine, diagnostic algorithm reported as a risk score for probability of rapid recurrence of recurrent or persistent cancer following transurethral resection Proprietary test: Oncuria® Predict Lab/Manufacturer: DiaCarta Clinical Lab
0420U	Oncology (urothelial), mRNA expression profiling by real-time quantitative PCR of MDK, HOXA13, CDC2, IGFBP5, and CXCR2 in combination with droplet digital PCR (ddPCR) analysis of 6 single-nucleotide polymorphisms (SNPs) genes TERT and FGFR3, urine, algorithm reported as a risk score for urothelial carcinoma.

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

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Revision History

Revision Date	Summary of Changes
03/06/2024	Reviewed and Updated: Updated the background, guidelines and recommendations, and evidence-based scientific references. Literature review did not necessitate any modifications to coverage criteria.
12/06/2023	Off-cycle coding modification: Added CPT code 0420U (new code effective 1/1/2024)
03/01/2023	Reviewed and Updated: Updated the background, guidelines and recommendations, and evidence-based scientific references. Literature review did not necessitate any modifications to coverage criteria. The following edits were made for clarity: Former CC3 and CC4 were wrapped into a single CC with subcriteria. Now reads: "3) For the evaluation of hematuria, to screen for bladder cancer in asymptomatic individuals, to diagnose bladder cancer in symptomatic individuals, or for any other indication not discussed above, the following tests DO NOT MEET COVERAGE CRITERIA: a) Urinary biomarkers (bladder tumor antigen (BTA) test, nuclear matrix protein (NMP22) test, or fluorescence in situ hybridization (FISH) UroVysion Bladder Cancer test). b) Fluorescence immunocytology (ImmunoCyt/uCyt)." Added CPT 0363U effective 1/1/2023, 0365U, 0366U, 0367U effective 4/1/2023
06/14/2022	Off-cycle coding change: Added CPT codes 88346, 88350.
03/09/2022	Annual review: Updated background, guidelines, and evidence-based scientific references. Literature review did not necessitate any modification to coverage criteria. Added the word "as" to the following CC for clarity: As an adjunct in the diagnostic exclusion of bladder cancer for patients who have an atypical or equivocal cytology Added CPT code 88346, 88350

03/03/2021	Annual review: Updated background, guidelines and recommendations, and evidence-based scientific references. Literature review did not necessitate any modification to coverage criteria.
03/10/2020	Annual review: Updated background, guidelines and recommendations, and evidence-based scientific references. Literature review did necessitate the following changes to CC: addition of "Any other urinary tumor markers for bladder cancer not mentioned above **DO NOT MEET COVERAGE CRITERIA".**
03/01/2019	Annual review: Updated definition, background, federal regulations, guidelines and recommendations, and evidence-based scientific references. Added the clarifier "high-risk, non-muscle invasive" to CC that allows for the use of urinary biomarkers as an adjunct to monitoring bladder cancer per NCCN v5.2018 guidelines. Added 0012M and 0013M (Cxbladder). Removed 88271, 88299 and 88365 (out of scope).
03/16/2018	Off-Cycle Review: Policy was reviewed to change the Annual Review Cycle. Literature review did not necessitate any modification to coverage criteria. No changes in coding.
09/28/2017	Annual review: Guidelines and Recommendations and Evidence-based Scientific References were updated. Literature review necessitated a change in CC based on NCCN 2017 and AUA 2016 guidelines. CPT coding updated: 86294, 86316, 86386, 88120, 88121 and 88271 changed from not covered to no PA required in accordance with new coverage criteria; CPT code 88365 added to policy as no PA required; changed 88229 from PA required to not covered
09/19/2016	Annual review completed. Literature review did not necessitate any modification to coverage criteria. References updated.
09/18/2015	Initial presentation

Revision Date	Summary of Changes
	CC1 edited for clarity and consistency. "Policy Guidelines" section replaced with Note 1 (signs of hypothyroidism) and Note 2 (signs of hyperthyroidism), former Note 1 on testing in pregnancy becomes Note 3. CC1.a.iv. frequency for hypothyroidism follow up changed from "6-12" to "every 6 weeks". Now reads: "iv) For individuals being treated for hypothyroidism, monitoring with TSH and fT4 testing every 6 weeks upon dosage change and annually in stable individuals." CC1b.v.a., frequency for hyperthyroidism follow up changed from "6-12" to "every 8 weeks". Now reads: "(a) In patients being treated for hyperthyroidism, repeat testing of TSH and fT4 should occur every 8 weeks." Former CC1.e. pertaining to thyroid testing has been replaced with new CC2 and CC3: "2) For individuals who are pregnant or who are postpartum and who have symptoms of thyroid dysfunction (see Note 1 and Note 2), TSH and fT4 testing (once every 4 weeks) MEETS COVERAGE CRITERIA (see Note 3). 3) For individuals who are pregnant or who are postpartum and who have been diagnosed with hyperthyroidism, total T4 (TT4), antithyroglobulin antibody (Tg-Ab), thyrotropin receptor antibodies (TRAb), and anti-thyroid peroxidase antibody (TPOAb) MEETS COVERAGE CRITERIA (see Note 3)." Thyroid antibody testing expanded beyond autoimmune thyroiditis, now allowing testing in hypothyroidism or hyperthyroidism, with testing restricted to once every 3 years. Former CC2, now CC4 reads: "4) For individuals with hypothyroidism or hyperthyroidism, testing for thyroid antibodies (once every three years) MEETS COVERAGE CRITERIA." **TBG added as not covered under any circumstances. Former CC4, now CC6 now reads: "6) For the evaluation of the cause of hyperthyroidism or hypothyroidism, testing for thyrotropin-releasing hormone (TRH) or thyroxine-binding globulin (TBG) DOES NOT MEET COVERAGE CRITERIA." **Added CPT 84442

Urine Culture Testing for Bacteria

Policy Number: AHS – G2156 – Urine Culture Testing for Bacteria	Prior Policy Name and Number, as applicable:
Initial Presentation Date: 7/27/2018 Revision Date: February 1, 2025	

Policy Description

Bacteriuria is the presence of bacteria in the urine. Urinary tract infections (UTIs) can occur in the urinary system and can be either symptomatic or asymptomatic. UTIs can include cystitis, an infection of the bladder or lower urinary tract; pyelonephritis, an infection of the upper urinary tract or kidney; urosepsis; urethritis; and male-specific conditions, such as bacterial prostatitis and epididymitis (Bonkat et al., 2023; Hooton & Gupta, 2023). Typically, in an infected person, bacteriuria, and pyuria (the presence of pus in the urine) are present and can be present in both symptomatic and asymptomatic UTIs. A urine culture can be performed to determine the presence of bacteria and to characterize the bacterial infection (Meyrier, 2023).

For guidance on pathogen panel testing from urine samples, please see AHS-G2149 Pathogen Panel Testing.

Related Policies

Policy Number	Policy Title
AHS-G2149	Pathogen Panel Testing

Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) For pregnant individuals, urine culture testing (with isolate identification and antibiotic susceptibilities if applicable) for a urinary tract infection (UTI) **MEETS COVERAGE CRITERIA**.

2) For asymptomatic individuals undergoing urological interventions which breach the mucosa, urine culture testing (with isolate identification and antibiotic susceptibilities if applicable) prior to the procedure **MEETS COVERAGE CRITERIA**.

3) For individuals exhibiting at least one sign or symptom of a possible UTI or bacteriuria (see Note 1 below), urine culture testing (with isolate identification and antibiotic susceptibilities if applicable) **MEETS COVERAGE CRITERIA**.

4) To assess pyelonephritis, urine culture testing (with isolate identification and antibiotic susceptibilities if applicable) **MEETS COVERAGE CRITERIA**.

5) For all other instances of asymptomatic UTI or asymptomatic bacteriuria not described above, urine culture testing (with isolate identification and antibiotic susceptibilities if applicable) **DOES NOT MEET COVERAGE CRITERIA**.

6) For individuals that show evidence of clinical resolution of infection, follow-up urine culture testing for an uncomplicated UTI **DOES NOT MEET COVERAGE CRITERIA**.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

7) Urine culture testing (with isolate identification and antibiotic susceptibilities if applicable) **DOES NOT MEET COVERAGE CRITERIA** in **any** of the following situations:
   a) As a part of initial screening for asymptomatic prostatitis.
   b) As a part of assessment or prognosis of prostate biopsy.

NOTES:

Note 1: Signs and symptoms of UTI/bacteriuria include (CDC, 2021)

- Fever
- Urgency to urinate
- Feeling the need to urinate despite having an empty bladder
- Increased frequency of urination
- Dysuria
- Suprapubic tenderness
- Pyuria
- Hematuria

- Cloudy urine
- Lower Back and Side (flank) pain
- Nausea
- Vomiting
- Chills
- Night sweats
- Pelvic pressure
- Change in urine smell
- Abnormal urinalysis findings

Table of Terminology

Term	Definition
AAP	American Academy of Pediatrics
ABIM	American Board of Internal Medicine
ABP	Acute bacterial prostatitis
ACOG	American College of Obstetricians and Gynecologists
AMDA	The Society for Post-Acute and Long-Term Care Medicine
ARESC	Antimicrobial Resistance Epidemiological Survey on Cystitis
ASB	Asymptomatic bacteriuria
ASPN	American Society of Pediatric Nephrology
AUA	American Urological Association
AUC	Area under the curve
BP	Bacterial prostatitis
CAUTI	Catheter-associated urinary tract infection
CBP	Chronic bacterial prostatitis
CDC	Centers for Disease Control and Prevention
CFU	Colony-forming unit
CLIA '88	Clinical Laboratory Improvement Amendments of 1988
CMS	Centers For Medicare and Medicaid
CPS	Canadian Paediatric Society
CUA	Canadian Urological Association
cUTI	Complicated urinary tract infection
DNA	Deoxyribonucleic acid
DOR	Diagnostic odds ratio
EAU	European Association of Urology
EQUC	Enhanced quantitative urine culture
FDA	Food and Drug Administration
FUM	Female urinary microbiota
ICU	Intensive care unit
IDSA	Infectious Diseases Society of America
KT	Kidney transplant
LCD	Local coverage determination
LDT	Laboratory-developed test
MRSA	Methicillin-Resistant *Staphylococcus Aureus*
MSSA	Methicillin-Sensitive *Staphylococcus Aureus*
NAAT	Nucleic acid amplification test

Term	Definition
NCD	National coverage determination
NICE	National Institute for Health and Care Excellence
NSQIP	National Surgical Quality Improvement Program
OR	Odds ratio
PA	Prior authorization
PCR	Polymerase chain reaction
RNA	Ribonucleic acid
rUTIs	Recurrent urinary tract infection
SCI	Spinal cord injury
SHEA	Society for Healthcare Epidemiology of America
SOT	Solid organ transplant
SPA	Suprapubic aspiration
SSI	Surgical site infection
SUFU	Society Of Urodynamics, Female Pelvic Medicine & Urogenital Reconstruction
UA	Urinalysis
USPSTF	United States Preventive Services Task Force
UTI	Urinary tract infection
WHO	World Health Organization

Scientific Background

Urinary tract infections (UTIs) can be either symptomatic or asymptomatic and can be classified as uncomplicated or complicated. Uncomplicated UTIs are "acute, sporadic or recurrent cystitis limited to non-pregnant women with no known relevant anatomical and functional abnormalities within the urinary tract or comorbidities" (Bonkat et al., 2023). All other UTIs that are not defined as uncomplicated are complicated UTIs. Complicated UTIs include "UTIs in a patient with an increased chance of a complicated course: i.e. all men, pregnant [individuals], patients with relevant anatomical or functional abnormalities of the urinary tract, indwelling urinary catheters, renal diseases, and/or with other concomitant immunocompromising diseases for example, diabetes" (Bonkat et al., 2023). *Escherichia coli* is the most common cause of complicated UTIs; however, "other uropathogens include other Enterobacteriaceae (such as *Klebsiella* spp and *Proteus* spp), *Pseudomonas*, enterococci, and staphylococci (methicillin-sensitive *Staphylococcus aureus* [MSSA] and methicillin-resistant *S. aureus* [MRSA])" (Hooton & Gupta, 2023). Even though both bacteriuria and pyuria are often present in UTIs, their presence alone is not indicative of a symptomatic infection.

The presence of bacteriuria does not guarantee negative outcomes for a patient. In fact, the paradigm of the sterility of the bladder environment has changed over recent years. At least for females, the presence of female urinary microbiota (FUM) is believed to occur naturally and has been documented using sensitive bacterial DNA screening tests on asymptomatic females (Brubaker & Wolfe, 2016). Beneficial microbes, such as vaginal strains of *Lactobacillus*, can inhibit the grown of uropathogenic bacteria, including *E. coli* (Aroutcheva et al., 2001; Brubaker & Wolfe, 2016). Over-prescribing antibiotics, especially in cases of asymptomatic bacteriuria, can lead to both an eradication of beneficial bacterial flora and an emergence of antibiotic-resistant bacteria. Prescribing antibiotics as a prophylactic measure or in the instance of asymptomatic bacteriuria is detrimental because it is of limited value and can also increase incidences of drug-resistance. A study in 2002 by Harding and colleagues show that antibiotic treatment in diabetic women with asymptomatic bacteriuria did not result in a decrease of future symptomatic UTIs as compared to the control group; in fact, the experimental group had higher rates of

adverse antimicrobial reactions (Harding et al., 2002). Even though the evidence-based guidelines by various societies, such as the EAU (Bonkat et al., 2023) and SHEA (SHEA, 2019), do not recommend performing urine testing or treatment for asymptomatic bacteriuria, inappropriate treatment is still occurring; in fact, one study by Cope and colleagues show that 32% of catheter-associated cases of asymptomatic bacteriuria and asymptomatic UTI received inappropriate treatment (Cope et al., 2009). The Antimicrobial Resistance Epidemiological Survey on Cystitis (ARESC) shows that up to 10.3% of *E. coli* in UTIs are "resistant to at least three different classes of antimicrobial agents" with ampicillin having the highest degree of resistance (48.3%). This is a large study of 4264 women from ten different countries to show that antibiotic-resistance is of international importance (Schito et al., 2009).

Analytical Validity

Urinalysis (UA) to detect nitrite and leukocyte esterase to indicate the presence of bacteria is an accepted laboratory practice. One report, though, has shown that the use of nitrite has "a sensitivity of 3%, a specificity of 97%, and a negative predictive value of 55%" (Cooper et al., 1992). A 2004 meta-analysis study asserts that the "sensitivities of the combination of both tests vary between 68 and 88% in different patient groups, but positive test results have to be confirmed" (Devillé et al., 2004). They did note that the accuracy of the leukocyte esterase testing was higher in urology patients with a diagnostic odds ratio (DOR) of 276 as compared to the accuracy of nitrites (for example, in elderly patients DOR = 108) (Devillé et al., 2004).

Urine culture is considered a "gold standard" for detecting the presence of bacteria in urine (Graham & Galloway, 2001; Schmiemann et al., 2010). That being said, "the interpretation of culture results can be considered as more of an art than a science. A urine culture result depends on so many variables, such as appropriate collection, transport, and the limits of the methods of detection. The reliability of single positive urine culture in diagnosing UTI is only 80%, rising to 90% if a repeat culture shows identical results" (Graham & Galloway, 2001). This is using the definition of bacteriuria as being 10^5 bacteria/ml of urine.

A potential future alternative to the urine culture could be multiplex PCR-based molecular testing, which Wojno et al. (2020) had found to be noninferior to urine culture for detection and identification of the bacteria. Agreement between the two testing methods was 90%, which exceeded the 85% noninferiority threshold. The multiplex PCR was also able to detect bacteria in 36% of symptomatic patients who had negative urine cultures and detected more polymicrobial infections than urine culture in a shorter amount of time (6 hours vs 48 hours for urine culture) (Wojno et al., 2020).

Clinical Utility and Validity

A study in 2010 by Bruyere et al. (2010) using 353 patients undergoing prostate biopsy show that the routine use of obtaining a pre-operative urine culture is not clinically relevant to positive outcomes. "Of the 353 men, 12 had a pre-biopsy-positive bacterial culture and underwent prostate biopsy without any infections complication. Fifteen patients with a negative pre-biopsy culture developed a post-biopsy-positive bacterial culture, but remained asymptomatic without any treatment. Only four men from the group without pre-biopsy bacteriuria developed an infectious complication, requiring 3 weeks of antibiotic therapy." Both experimental and control groups had similar rates of complication, suggesting "that routine urine bacterial culture before prostate biopsy is not useful when antibiotic prophylaxis and enema are performed" (Bruyere et al., 2010).

The method of obtaining the urine sample for culture testing is important. This is especially true for children. A 2017 study of 4808 acutely ill children demonstrated that there was modest agreement between the results obtained if the test was conducted by a research laboratory versus a health service laboratory; however, the method of obtaining the urine sample did have significance. The calculated areas under the receiver-operator curve (AUC) for UTI ranged from 0.75-0.86 if the sample was obtained using a clean-catch method versus AUC values of 0.65-0.79 if the sample was obtained using "nappy pad samples". The authors conclusions were that urine cultures did not necessarily have to be sent to a research lab for testing, but that "primary care clinicians should try to obtain clean catch samples, even in very young children" (Birnie et al., 2017). A smaller study of 83 infants compared the use of urine obtained either via bladder catheterization or suprapubic aspiration (SPA) (Eliacik et al., 2016). All 83 infants had previously tested positive using urine culture samples obtained via bladder catheterization. Then, they had samples removed by SPA. The SPA samples were used in both urinalysis and urine culture testing, and "only 24 (28.9%) and 20 (24%) yielded positive urine culture and abnormal urinalysis data, respectively." This indicates a 71.1% false-positive result rate if the urine sample is obtained using bladder catheterization. "In infants younger than 12 months, SPA is the best method to avoid bacterial contamination, showing better results than transurethral catheterization" (Eliacik et al., 2016).

Another study by Ducharme et al. (2007) researched the use of either urine cultures and/or reagent test strips for use in diagnosing UTIs in elderly patients. The study consisted of 100 elderly patients with one group having no symptoms and non-infectious complaints and a second group "presenting with acute confusion, weakness or fever but no apparent urinary symptoms". Their results show that "of the 33 positive cultures, 10 had negative reagent strips. Thirteen of the 14 positive nitrite tests were culture positive for a specificity of 92.8% and a sensitivity of 36.1%. Positive cultures did not infer a diagnosis of UTI. Of the 67 positive reagent strips, 41 (61.2%) were associated with negative cultures." They conclude that, "in the elderly, reagent testing is an unreliable method of identifying patients with positive blood cultures. Moreover, positive urine culture rates are only slightly higher in patients with vague symptoms attributable to UTI than they are in (asymptomatic) patients treated for non-urologic problems, which suggests that many positive cultures in elderly patients with non-focal systemic symptoms are false-positive tests reflecting asymptomatic bacteriuria and not UTIs" (Ducharme et al., 2007).

A study by Price et al. (2016)show that using an enhanced quantitative urine culture (EQUC) increased the detection of microorganisms in UTIs. This study consisted of 150 female patients using an initial UTI symptom assessment questionnaire to divide them into symptomatic and asymptomatic groups. Both sets underwent culture testing using both conventional urine culture testing and an EQUC method. "Compared to expanded-spectrum EQUC, standard urine culture missed 67% of uropathogens overall and 50% in participants with severe urinary symptoms. Thirty-six percent of participants with missed uropathogens reported no symptom resolution after treatment by standard urine culture results." Their protocol resulted in an "84% uropathogen detection relative to 33% detection by standard urine culture" (Price et al., 2016).

Cantey et al. (2015) evaluated the utility of a Gram stain relative to UA. In reviewing 312 pediatric patients with suspected UTIs who had urine cultures, UA, and Gram stain performed, the researchers concluded that the UA "has excellent negative predictive value that is not enhanced by urine Gram stain and that antibiotic selection did not vary based on the urine Gram stain result." When compared to the urine Gram stain, the UA had equal sensitivity (97.3% vs 97.5%) and a higher specificity (85% vs 74%). This could allow the UA to take precedent as a test performed over the Gram stain due to its increased efficiency and lower cost (Cantey et al., 2015).

Petty et al. (2019)evaluated the risk factors and clinical outcomes of treating asymptomatic bacteriuria (ASB) in hospitalized patients. 2733 patients with ASB (defined as "positive urine culture without any documented signs or symptoms attributable to urinary tract infection") were included. 2259 patients were treated with antibiotics for a mean of 7 days. Certain characteristics tended to correlate with ASB treatment, such as positive urinalysis (odds ratio [OR] = 2.83), leukocytosis (OR = 1.55), and dementia (OR = 1.57). However, treatment of ASB was found to be associated with longer duration of hospitalization after urine testing (4 vs 3 days; relative risk, 1.37), although no other differences in secondary outcomes were identified. The authors concluded that "hospitalized patients with ASB commonly receive inappropriate antibiotic therapy. Antibiotic treatment did not appear to be associated with improved outcomes; rather, treatment may be associated with longer duration of hospitalization after urine testing." The authors also recommended stewardship efforts to reduce inappropriate treatment (Petty et al., 2019).

Coussement et al. (2019) investigated the prevalence of asymptomatic bacteriuria among kidney transplant patients beyond two months post-transplant. The authors identified 500 post-transplant patients, of which 17 had asymptomatic bacteriuria (3.4%). Further, of the 76 patients that were 2-12 months post-transplant, only one had asymptomatic bacteriuria, and of the other 424 patients, 16 patients had asymptomatic bacteriuria. The authors concluded that the prevalence of asymptomatic bacteriuria past the second month of kidney transplant was low and that further studies were needed to ascertain the cost-effectiveness of the screen-and-treat strategy in this population (Coussement et al., 2019). This finding regarding screening and treating ASB was confirmed by Fontserè et al. (2021), who found that the "treatment of A[S]B diminished the microbiological cure and increased the rates of microbiologic relapses and reinfections… treated A[S]B patients showed a trend of developing symptomatic urinary tract infection in the following six months."

Guidelines and Recommendations

Choosing Wisely

Choosing Wisely, an initiative by the American Board of Internal Medicine (ABIM) Foundation, consists of several national organizations representing medical specialists that write recommendations within their respective field to help choose care based on scientific evidence and to help reduce testing redundancy.

2019 AMDA-The Society for Post-Acute and Long-Term Care Medicine (AMDA)

In 2019, the AMDA updated their earlier 2017 Choosing Wisely guideline concerning the use of urine cultures. Due to overuse of antibiotics and overtreatment of UTIs, they state "Don't obtain urine tests until clinical criteria are met." Since the urine culture would have a high likelihood of yielding a positive result in an otherwise asymptomatic case, this "contributes to the over-use of antibiotic therapy in this setting, leading to an increased risk of diarrhea or other adverse drug events, resistant organisms and infection due to *Clostridioides difficile*." They also note that "the finding of asymptomatic bacteriuria may lead to an erroneous assumption that a UTI is the cause of an acute change of status, hence failing to detect or delaying the timelier detection of 5 signs and symptoms likely indicative of uncomplicated cystitis. These include dysuria, and one or more of the following: frequency, urgency, supra-pubic pain or gross hematuria" (AMDA, 2019).

2018 American Academy of Pediatrics-Section on Nephrology (ASPN) and the American Society of Pediatric Nephrology (AAP)

The AAP Section on Nephrology and the ASPN issued a joint Choosing Wisely recommendation stating, "Avoid ordering follow-up urine cultures after treatment for an uncomplicated urinary tract infection (UTI) in patients that show evidence of clinical resolution of infection. Studies have shown that clinical resolution of infection is adequate for determining effectiveness of antibiotic therapy after treatment for a UTI" (AAP & ASPN, 2018).

2016 American Academy of Pediatrics (AAP)

The AAP updated their Choosing Wisely recommendation in 2016: "Avoid the use of surveillance cultures for the screening and treatment of asymptomatic bacteriuria." There is no evidence that surveillance urine cultures or treatment of asymptomatic bacteriuria is beneficial. Surveillance cultures are costly and produce both false positive and false negative results. Treatment of asymptomatic bacteriuria is harmful and increases exposure to antibiotics, which is a risk factor for subsequent infections with a resistant organism. This also results in the overall use of antibiotics in the community and may lead to unnecessary imaging" (AAP, 2016).

2019 Society for Healthcare Epidemiology of America (SHEA)

The SHEA recommendation in Choosing Wisely is more encompassing: "Don't perform cultures (e.g. urine, blood, sputum cultures) or test for *C. difficile* unless patients have signs or symptoms of infection. Tests can be falsely positive leading to over diagnosis and overtreatment. Although important for diagnosing disease when used in patients with appropriate signs or symptoms, these tests often are positive when an infection is not present. For example, in the absence of signs or symptoms, a positive blood culture may represent contamination, a positive urine culture could represent asymptomatic bacteriuria, and a positive test for *C. difficile* could reflect colonization. There are no perfect tests for these or most infections. If these tests are used in patients with low likelihood of infection, they will result in more false positive tests than true positive results, which will lead to treating patients without infection and exposing them to risks of antibiotics without benefits of treating an infection" (SHEA, 2019).

European Association of Urology (EAU)

The EAU has guidelines for urological infections that are updated annually. With respect to **asymptomatic bacteriuria**, they state (all with a 'Strong' strength of rating), "Do not screen or treat asymptomatic bacteriuria in the following conditions:

- Women without risk factors;
- Patients with well-regulated diabetes mellitus;
- Post-menopausal [individuals];
- Elderly institutionalised patients;
- Patients with dysfunctional and/or reconstructed lower urinary tracts;
- Patients with renal transplants;
- Patients prior to arthroplasty surgeries;
- Patients with recurrent urinary tract infections."

They do recommend with a 'Strong' rating to "screen for and treat asymptomatic bacteriuria prior to urological procedures breaching the mucosa" and a 'Weak' rating to "screen for and treat asymptomatic bacteriuria in pregnant [individuals] with standard short course treatment." They do recommend to "diagnose **recurrent UTI** by urine culture" with a 'Strong' rating. Please note that recurrent UTI indicates

that the occurrences are symptomatic. It is further specified that "A urine culture must therefore be taken prior to such interventions".

With respect to **uncomplicated cystitis**, they give a 'Strong' rating to only perform urine culture analysis "in the following situations:

- Suspected acute pyelonephritis;
- Symptoms that do not resolve or recur within four weeks after the completion of treatment;
- Women who present with atypical symptoms;
- Pregnant [individuals]."

The EAU gives a 'Weak' recommendation to "use urine dipstick testing for diagnosis of acute uncomplicated cystitis."

In cases of uncomplicated **pyelonephritis**, the EAU recommends with a 'Strong' rating to "perform urinalysis (e.g. using the dipstick method), including the assessment of white and red blood cells and nitrite, for routine diagnosis" and to "perform urine culture and antimicrobial susceptibility testing in patients with pyelonephritis."

The EAU defines **complicated UTI** (cUTI) as occurring "in an individual in whom factors related to the host (e.g. underlying diabetes or immunosuppression) or specific anatomical or functional abnormalities related to the urinary tract (e.g. obstruction, incomplete voiding due to detrusor muscle dysfunction) are believed to result in an infection that will be more difficult to eradicate than an uncomplicated infection." Other factors associated with cUTIs include vesicoureteral reflux, recent history of instrumentation, UTI in males, pregnancy, and healthcare-associated infections. "Laboratory urine culture is the recommended method to determine the presence or absence of clinically significant bacteriuria in patients suspected of having a cUTI".

For **catheter-associated UTIs** (CAUTI), the EAU recommends with 'Strong' ratings to "not carry out routine urine culture in asymptomatic catheterised patients", to "not use pyuria as sole indicator for catheter-associated UTI", and to "not use the presence or absence of odorous or cloudy urine alone to differentiate catheter-associated asymptomatic bacteriuria from catheter-associated UTI."

In cases of **urethritis,** the EAU states that "Clinicians should always perform point-of-care diagnostics (e.g. Gram staining, first-void urine with microscopy, leukocyte esterase testing) if available to obtain objective evidence of urethral inflammation and to guide treatment…men who meet the criteria for urethritis should be tested for *C. trachomatis, M. genitalium* and *N. gonorrhoeae* with nucleic acid amplification tests (NAAT), even if point-of-care tests are negative for gonorrhoeae…*N. gonorrhoeae* and chlamydia cultures are mainly to evaluate treatment failures and monitor developing resistance to current treatment." With a 'Strong' rating, they recommend:

- "Perform a gram stain of urethral discharge or a urethral smear to preliminarily diagnose gonococcal urethritis."
- "Perform a validated nucleic acid amplification tests on a first-void urine sample or urethral smear to prior to empirical treatment to diagnose chlamydial and gonococcal infections."
- "Perform a urethral swab culture, prior to initiation of treatment, in patients with a positive NAAT for gonorrhoea to assess the antimicrobial resistance profile of the infective strain."
- "Use a pathogen directed treatment based on local resistance data."

For **urosepsis,** the EAU strongly recommends to "Take a urine culture and two sets of blood cultures before starting antimicrobial treatment."

For the diagnosis and disease management of **bacterial prostatitis** (BP), the EAU recommends with a 'Strong' rating to "perform the Meares and Stamey 2- or 4-glass test in patients with [chronic bacterial prostatitis (CBP)]". They only give a 'Weak' rating in the use of the urine dipstick test and blood culture with a total blood count for acute bacterial prostatitis (ABP). They also give a 'Weak' rating to their recommendation to "not routinely perform microbiological analysis of the ejaculate alone to diagnose CBP"; however, they give a 'Strong' recommendation to "treat acute bacterial prostatitis according to the recommendations for complicated UTIs" where they recommend a laboratory urine culture.

The EAU's recommendation in cases of suspected **acute infective epididymitis** (with a 'Strong' rating) is "to obtain a mid-stream urine and a first-voided urine for pathogen identification by culture and nucleic acid amplification test." It should be noted that, if the acute scrotal pain and/or swelling is due to suspected torsion, then a urine culture is not necessary. In that case, "urgent surgical exploration" is recommended instead (Bonkat et al., 2023).

World Health Organization (WHO)

The *WHO recommendations on antenatal care for a positive pregnancy experience* in 2016 does include a recommendation to test for asymptomatic bacteriuria (ASB) in pregnant individuals. "Midstream urine culture is the recommended method for diagnosing asymptomatic bacteriuria (ASB) in pregnancy. In settings where urine culture is not available, the onsite midstream urine Gram-staining is recommended over the use of dipstick tests as the method for diagnosing ASB in pregnancy." They do make note of the amount of time a urine culture takes (up to 7 days) but state that it is "the gold standard". The concern of ASB in pregnancy is because "ASB is associated with an increased risk of preterm birth" (WHO, 2016).

Canadian Paediatric Society (CPS)

In 2014, the CPS issued their position statement titled *Urinary tract infection in infants and children: Diagnosis and management* and reaffirmed their statement in 2020. Their recommendations are for children >2 months old. They recommend that "infants from two to 36 months of age with a fever of >39°C and no other source for fever on history or physical examination…should have urine collected for urinalysis. Unless this test is completely normal, they should then have urine collected by catheter or suprapubic aspirate [SPA] sent for culture." Currently, CPS notes this statement as inapplicable for infants under 2 months of age (Robinson et al., 2020).

If the child has been toilet-trained, then the urine sample can be collected midstream in lieu of the catheter. "Children with possible UTI who require antibiotic treatment immediately for other indications, such as suspected bacteremia, should have urine collected for urinalysis, microscopy, and culture." Again, this sample should be obtained via either catheterization or SPA unless the child has been toilet-trained. They also state that "urine collection must occur before starting antibiotics because a single dose of an effective antibiotic rapidly sterilizes the urine" (Robinson et al., 2020).

American Academy of Pediatrics (AAP)

The AAP issued guidelines for UTIs in children 2 to 24 months of age in 2011, which were reaffirmed in 2016. With an "A" grade for evidence quality and a strong recommendation, they issued their Action Statement 1: "If a clinician decides that a febrile infant with no apparent source for the fever requires

antimicrobial therapy to be administered because of ill appearance or another pressing reason, the clinician should ensure that a urine specimen is obtained for both culture and urinalysis before an antimicrobial agent is administered; the specimen needs to be obtained through catheterization or SPA, because the diagnosis of UTI cannot be established reliably through culture of urine collected in a bag." For instances where the clinician believes that the febrile child does not warrant immediate antimicrobial therapy, the AAP in Action Statement 2 (strong recommendation; "A" grade of evidence) the following: (Action Statement 2a) "If the clinician determines the febrile infant to have a low likelihood of UTI [in Table below] then the clinical follow-up monitoring without testing is sufficient." In Action Statement 2b, the AAP states: "If the clinician determines that the febrile infant is not in a low-risk group [in Table below], then there are 2 choices. Option 1 is to obtain a urine specimen through catheterization or SPA for culture and urinalysis. Option 2 is to obtain a urine specimen through the most convenient means and to perform a urinalysis. If the urinalysis results suggest a UTI (positive leukocyte esterase test results or nitrite test or microscopic analysis results positive for leukocytes or bacteria), then a urine specimen should be obtained through catheterization or SPA and cultures; if urinalysis of fresh (<1 hour since void) urine yields negative leukocyte esterase and nitrite test results, then it is reasonable to monitor the clinical course without initiating anti-microbial therapy, recognizing that negative urinalysis results do not rule out a UTI with certainty." The table below from (Roberts, 2011) depicts the level of risk factors separated by gender.

Individual Risk Factors: Girls
White race Age < 12 mo Temperature ≥ 39°C Fever ≥ 2 d Absence of another source of infection

Probability of UTI	No. of Factors Present
≤1%	No more than 1
≤2%	No more than 2

Individual Risk Factors: Boys
Nonblack race Temperature ≥ 39°C Fever > 24 h Absence of another source of infection

Probability of UTI	No. of Factors Present	
	Uncircumcised	Circumcised
≤1%	[a]	No more than 2
≤2%	None	No more than 3

FIGURE 2
Probability of UTI Among Febrile Infant Girls[28] and Infant Boys[30] According to Number of Findings Present. [a]Probability of UTI exceeds 1% even with no risk factors other than being uncircumcised.

Canadian Urological Association (CUA)

The CUA *Guidelines for the diagnosis and management of recurrent urinary tract infection in women* contains an algorithm for a "female without a prior history of structural or functional abnormalities of the urinary tract presenting with 3 or more UTIs in 12 months" that requires a urine culture during a time when the patient is symptomatic followed by a urine culture two weeks after initiating treatment with sensitivity-adjusted antibiotics (Level 4 evidence, Grade C recommendation [Recommendation 2c]). In doing so, this "may aid in confirming the diagnosis of UTI, as well as guiding further specialist evaluation and management." For recurrent uncomplicated UTI, "culture and sensitivity analysis should be performed at least once while the patient is symptomatic…. A midstream urine bacterial count of 1 X 10^5 CFU/L should be considered a positive culture while the patient is symptomatic." For patients that choose an option of 'self-start antibiotic' therapy, "it is not necessary to culture the urine after UTI self-diagnosis since there is a 86% to 92% concordance between self-diagnosis and urine culture in an appropriately selected patient population. Patients are advised to contact a health care provider if symptoms do not resolve within 48 hours for treatment based on culture and sensitivity" (Dason et al., 2011).

American Urological Association (AUA)

The AUA issued a white paper in 2014 concerning CAUTIs. In the white paper, they refer to the use of the National Surgical Quality Improvement Program (NSQIP) definition of UTIs, which does reference the use of urine culture. It should be noted, however, that this definition requires at least a minimum of one of the following symptoms: fever (>38°C), urgency, frequency, dysuria, or suprapubic tenderness. They too refer to the 2009 IDSA guidelines concerning CAUTIs as well as those of the EAU. They state that there are "no consistent guidelines are available on how to obtain urine for culture from chronically catheterized patients, or what constitutes true urinary tract infection versus asymptomatic bacteriuria." They make note of a study concerning the possible cost-effectiveness of the use of dipsticks to screen asymptomatic ICU patients for CAUTIs. They conclude, "however, as previously discussed, screening of asymptomatic patients may not be warranted, and treatment is usually not recommended in these cases" (Averch et al., 2014).

The AUA released guidelines for primary vesicoureteral reflux in children and recommend "Urinalysis for proteinuria and bacteriuria is recommended. If the urinalysis indicates infection, a urine culture and sensitivity is recommended". The AUA also recommends urinalysis annually as part of the follow-up procedure (AUA, 2017).

The AUA published an update to their 2012 guideline on Urologic Procedures and Antimicrobial Prophylaxis, termed a "Best Practice Statement." The AUA recommends that "Prior to any urologic procedure, evaluation of a patient's urinary tract symptoms suggestive of a UTI should include a simple dipstick, laboratory performed microscopy, and/or formal culture." The AUA also states that "Positive microscopy findings should be confirmed with a culture for antimicrobial sensitivities in the perioperative setting where the risk of an SSI is high and targeted antimicrobial treatment may be required. Urine culture should not be performed without an accompanying urine microscopy due to common sample contamination as well as bacterial colonization" (Lightner et al., 2020).

National Institute for Health and Care Excellence (NICE)

In 2023, the NICE updated their quality standards for urinary tract infections in adults. They released five quality statements:

- "Statement 1: Women aged under 65 years are diagnosed with a urinary tract infection (UTI) if they have 2 or more key urinary symptoms and no other excluding causes or warning signs.
- Statement 2: Adults with indwelling urinary catheters do not have dipstick testing to diagnose UTIs.
- Statement 3: Men and non-pregnant women are not prescribed antibiotics to treat asymptomatic bacteriuria.
- Statement 4: Non-pregnant women with an uncomplicated lower UTI are prescribed a 3-day course of antibiotics, and men and pregnant women with an uncomplicated lower UTI are prescribed a 7-day course of antibiotics.
- Statement 5: Men with a recurrent UTI, and women with a recurrent lower UTI where the cause is unknown or a recurrent upper UTI are referred for specialist advice. (NICE, 2023).

NICE also recommended the following populations of children for a urine culture:

- in infants and children who are suspected to have acute pyelonephritis/upper urinary tract infection
- in infants and children with a high to intermediate risk of serious illness

- in infants under 3 months
- in infants and children with a positive result for leukocyte esterase or nitrite
- in infants and children with recurrent UTI
- in infants and children with an infection that does not respond to treatment within 24–48 hours, if no sample has already been sent
- when clinical symptoms and dipstick tests do not correlate (NICE, 2018).

American Urological Association (AUA)/Canadian Urological Association (CUA)/Society of Urodynamics, Female Pelvic Medicine & Urogenital Reconstruction (SUFU)

The AUA, CUA, and SUFU released joint guidelines in 2019. The guidelines were reviewed, and validity confirmed in 2022. These joint guidelines focus on "recurrent episodes of uncomplicated cystitis in women" and are not intended for "pregnant [individuals], patients who are immunocompromised, those with anatomic or functional abnormalities of the urinary tract, women with rUTIs due to self-catheterization or indwelling catheters, or those exhibiting signs or symptoms of systemic bacteremia, such as fever and flank pain". Their recommendations are listed below:

- "Clinicians should obtain urinalysis, urine culture and sensitivity with each symptomatic acute cystitis episode prior to initiating treatment in patients with rUTIs. (Moderate Recommendation; Evidence Level: Grade C)"
- "Clinicians should omit surveillance urine testing, including urine culture, in asymptomatic patients with rUTIs" (Moderate Recommendation; Evidence Level: Grade C)" (Anger et al., 2019).

Infectious Diseases Society of America (IDSA)

These 2019 guidelines were intended to update the 2005 IDSA guidelines. Their recommendations for asymptomatic bacteriuria (ASB) are as follows:

- "In infants and children, we recommend against screening for or treating asymptomatic bacteriuria".
- "In healthy premenopausal, nonpregnant [individuals] or healthy postmenopausal [individuals], we recommend against screening for or treating ASB".
- "In pregnant [individuals], we recommend screening for and treating ASB".
- "In older, community-dwelling persons who are functionally impaired, we recommend against screening for or treating ASB".
- "In older persons resident in long-term care facilities, we recommend against screening for or treating ASB".
- "In patients with diabetes, we recommend against screening for or treating ASB".
- "In renal transplant recipients who have had renal transplant surgery >1 month prior, we recommend against screening for or treating ASB".
- "In patients with nonrenal solid organ transplant (SOT), we recommend against screening for or treating ASB".
- "In patients with high-risk neutropenia (absolute neutrophil count <100 cells/mm3, ≥7 days' duration following chemotherapy), we make no recommendation for or against screening for or treatment of ASB".
- "In patients with spinal cord injury (SCI), we recommend against screening for or treating ASB".
- "In patients with a short-term indwelling urethral catheter (<30 days), we recommend against screening for or treating ASB".

- "In patients undergoing elective nonurologic surgery, we recommend against screening for or treating ASB".
- "In patients who will undergo endoscopic urologic procedures associated with mucosal trauma, we recommend screening for and treating ASB prior to surgery".

The guideline also states that it has been reviewed and endorsed by the following societies: "the Society of Healthcare Epidemiology of America, Pediatric Infectious Diseases Society, American College of Obstetrics and Gynecology, Association of Medical Microbiology and Infectious Diseases Canada, European Society of Clinical Microbiology and Infectious Diseases, European Association of Urology, and the American Urological Association" (Nicolle et al., 2019).

US Preventive Services Task Force (USPSTF)

The USPSTF recommends screening for "asymptomatic bacteriuria using urine culture in pregnant persons", but recommends against "screening for asymptomatic bacteriuria in nonpregnant adults" (USPSTF, 2019).

American Society of Transplantation Infectious Diseases

These guidelines focus on UTIs within the kidney transplant (KT) population. The recommendations are listed below:

"We recommend against routinely collecting urine culture or treating bacteriuria in asymptomatic KT patients more than two months after KT".

"If screening asymptomatic KT recipients any time in the post-transplant period and A[S]B [asymptomatic bacteriuria] is found, a second urine culture (minimizing risk of contamination) should be collected and reviewed prior to decision about whether or not to treat AB. We strongly recommend observation without treatment of asymptomatic KT patients recipients who show clearance of the initial bacteriuria or development of different organism in the urine" (Goldman & Julian, 2019).

Choosing Wisely Canada

The Association of Medical Microbiology and Infectious Diseases Canada recommends against collecting "urine specimens for culture from adults who lack symptoms localizing to the urinary tract or fever unless they are pregnant or undergoing genitourinary instrumentation where mucosal bleeding is expected." The guideline further recommends that laboratories "consider supplementing educational efforts to reduce collection of urine cultures from asymptomatic patients with analytical interventions that reduce processing of low-value specimens" (Association of Medical Microbiology and Infectious Diseases Canada, 2021).

Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
87077	Culture, bacterial; aerobic isolate, additional methods required for definitive identification, each isolate
87086	Culture, bacterial; quantitative colony count, urine
87088	Culture, bacterial; with isolation and presumptive identification of each isolate, urine
87140	Culture, typing; immunofluorescent method, each antiserum
87147	Culture, typing; immunologic method, other than immunofluorescence (eg, agglutination grouping), per antiserum
87149	Culture, typing; identification by nucleic acid (DNA or RNA) probe, direct probe technique, per culture or isolate, each organism probed
87181	Susceptibility studies, antimicrobial agent; agar dilution method, per agent (eg, antibiotic gradient strip)
87186	Susceptibility studies, antimicrobial agent; microdilution or agar dilution (minimum inhibitory concentration [MIC] or breakpoint), each multi-antimicrobial, per plate

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

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Revision History

Revision Date	Summary of Changes
05/31/2023	Reviewed and Updated: Updated the background, guidelines and recommendations, and evidence-based scientific references. Literature review did not necessitate any modifications to coverage criteria. The following edits were made for clarity: All CCs edited for clarity and consistency. Added CPT code 87186.

Venous and Arterial Thrombosis Risk Testing

Policy Number: AHS – M2041 – Venous and Arterial Thrombosis Risk Testing	Prior Policy Name and Number, as applicable: • AHS – M2041 – Venous Thrombosis Risk Testing
Initial Presentation Date: 06/16/2015 Effective Date: February 1, 2025	

Policy Description

A thrombosis, also known as a blood clot, occurs within blood vessels in the body. The two main types of thrombosis include venous thrombosis, which is when a vein is blocked due to a blood clot, and arterial thrombosis, which is when an artery is blocked due to a blood clot. Thrombophilias refer to hereditary and/or acquired abnormalities of hemostasis that predispose patients to thrombosis (Stevens et al., 2016). The most common presentations of venous thromboembolism (VTE) are deep vein thrombosis (DVT) and pulmonary embolism (PE) (Bartholomew, 2017).

Terms such as male and female are used when necessary to refer to sex assigned at birth.

Related Policies

Policy Number	Policy Title
AHS-M2141	Testing of Homocysteine Metabolism-Related Conditions

Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) For individuals without recurrent venous thromboembolism (VTE) risk factors (e.g., surgery, prolonged immobilization, collagen vascular disease, malignancy, certain hematologic disorders), plasma testing for protein C deficiency, protein S deficiency, and antithrombin III deficiency (see Note 1 and Note 2) **MEETS COVERAGE CRITERIA** in **any** of the following situations:
    a) For individuals less than 50 years of age who have experienced any deep venous thrombosis (DVT) or pulmonary embolism (PE).
    b) For individuals who have experienced a DVT in unusual sites (e.g., hepatic, mesenteric, or cerebral veins).
    c) For individuals who have experienced a DVT and who have a strong family history of thrombotic disease.
    d) For individuals who are pregnant or taking oral contraceptives and who have experienced a DVT.
    e) For first- and second-degree relatives (see Note 3) of individuals who experienced a DVT before 50 years of age.
    f) For women under the age of 50 who smoke and who have suffered a myocardial infarction.
    g) Before the administration of oral contraceptives, targeted testing of individuals with a personal or family history of DVT.
    h) For pediatric individuals who have suffered from a pediatric arterial ischemic stroke.
2) For individuals with warfarin-induced skin necrosis or for infants who develop neonatal purpura fulminans, plasma testing for protein C deficiency and protein S deficiency (see Note 1) **MEETS COVERAGE CRITERIA**.
3) Venous thrombosis risk testing for superficial venous thrombosis (including superficial thrombophlebitis and varicosities) **DOES NOT MEET COVERAGE CRITERIA**.
4) For all situations, activated protein C (aPC) resistance assay **DOES NOT MEET COVERAGE CRITERIA**.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

5) DVT risk testing as part of a pre-transplant evaluation test DOES NOT MEET COVERAG**E CRITERIA**.

NOTES:

Note 1: Plasma testing for protein C deficiency, protein S deficiency, and antithrombin III deficiency should be performed at least six weeks after the acute thrombotic event and while the patient is not taking anticoagulants. Assays for clotting inhibitors amount and function should be performed prior to any molecular testing.

Note 2: In addition to plasma testing (protein C deficiency, protein S deficiency, antithrombin III deficiency), risk factor testing for individuals suspected of having a hereditary and/or acquired thrombophilia should include genetic testing for Factor V Leiden and Prothrombin gene G20210A mutations.

Note 3: First-degree relatives include parents, full siblings, and children of the individual. Second-degree relatives include grandparents, aunts, uncles, nieces, nephews, grandchildren, and half-siblings of the individual.

Table of Terminology

Term	Definition
ACC	American College of Cardiology
ACMG	American College of Medical Genetics and Genomics
ACOG	American College of Obstetricians and Gynecologists
ACR	American College of Radiology
AHA/ASA	American Heart Association/American Stroke Association
aPC	Activated protein C
APS	Antiphospholipid syndrome
ASCP	American Society for Clinical Pathology
ASH	American Society of Hematology
COC	Combined oral contraceptives
CTPA	Computed tomography pulmonary angiography
DVT	Deep vein thrombosis
EGAPP	Evaluation of genomic applications in practice and prevention
ESC	European Society of Cardiology
FVL	Factor V Leiden
HRT	Hormone replacement therapy
NAFT	The North American Thrombosis Forum
OC	Oral contraceptives
PE	Pulmonary embolism
PGM	Prothrombin 20210A gene mutation
SIGN	Scottish Intercollegiate Guidelines Network
SPESI	Simplified pulmonary embolism severity index
SVM	Society for Vascular Medicine
VQ	Ventilation perfusion
VTE	Venous thromboembolism
VUS	Variant of unknown significance

Scientific Background

A thrombus is "an aggregate of coagulated blood within the vascular system or heart which contains platelets, fibrin, leukocytes, and red blood cells in varying amounts" (Herrmann, 2018). This aggregate of blood can be problematic as it may obstruct normal blood circulation throughout the body and even travel to peripheral areas. The primary manifestations of venous thromboembolisms (VTE) are deep vein thrombosis and pulmonary embolism. These conditions affect an estimated one million individuals in the United States annually (Bartholomew, 2017).

Thrombosis is widely theorized to develop due to Virchow's Triad, which consists of abnormalities in blood flow, a vascular endothelial injury, and alterations in the blood constituents. Changes in any of these characteristics may cause the clot to form (Bauer & Lip, 2024). For example, sickle red blood cells may cause increased clumping or decreased adhesion to the vessel walls (Byrnes & Wolberg, 2017). There are two main types of thrombosis: venous thrombosis (when a vein is blocked due to a blood clot) and arterial thrombosis (when an artery is blocked due to a blood clot).

A deep vein thrombosis (DVT) refers to a thrombus in a "deep" vein whereas a pulmonary embolism (PE) refers to an obstruction of the pulmonary artery (or one of its branches) by foreign material (Bauer, 2024a; Thompson, 2023). DVT of the lower extremities may cause symptoms, such as swelling or edema in the lower extremities, pain, and warmth in the affected area (Bauer, 2024a). This thrombus may travel to the lungs (becoming an embolus) and cause a PE. A PE has similar symptoms to DVT but may include pulmonary issues, such as shortness of breath. The risk factors for VTE, PE, and DVT are similar (Thompson, 2023). The two primary categories of risk factors for VTE are hereditary and acquired, and the genetic tendency toward VTE is referred to as inherited thrombophilia. Hereditary risk factors include genetic mutations such as Factor V Leiden (FVL) mutations. The five most common genetic risk factors for VTE are FVL mutations, prothrombin mutations, protein S defect, protein C defect, and antithrombin defect (Bauer & Lip, 2024). Approximately 50–60% of the variance in VTE incidence are attributed to genetic effects (Crous-Bou et al., 2016).

A modified activated partial thromboplastin time (aPTT) assay detects the anticoagulant activity of activated protein C (aPC). FVL mutations cause coagulation factor V to be unresponsive to aPC and initially, these changes were termed "aPC resistance" due to the reduced activity of aPC on a modified aPTT assay. A single nucleotide change (G1691A) results in a point mutation of glutamine to arginine at position 506. Approximately 99% of carriers of this mutation are heterozygous, and only 5% of these heterozygotes will experience a VTE in their lifetime. These mutations are often suspected in patients experiencing a VTE at a young age (under 50), a VTE in unusual areas such as a portal vein, or recurrent VTEs (Bauer, 2023a). Protein C may also be genetically deficient, but this mutation is only seen in 2-5% of individuals with a VTE (Bauer, 2024a). Protein S, a cofactor for the aPC control mechanism, and deficiencies in this protein may also confer additional risk for VTE (Bauer, 2024b).

The second most common inherited thrombophilia is the G20210A mutation of prothrombin. This mutation is a gain of function mutation where clotting activity is increased by creating more thrombin and fibrin. The overall prevalence of this mutation is about 2% (Bauer, 2023c). Genetic defects of antithrombin (an inhibitor of thrombin) may also occur, but the estimated prevalence of antithrombin defects is only a maximum of 0.2% (Bauer, 2023b).

Acquired risk factors or predisposing conditions for thrombosis include a prior thrombotic event, recent major surgery, presence of a central venous catheter, trauma, immobilization, malignancy, pregnancy, the use of oral contraceptives or heparin, myeloproliferative disorders, antiphospholipid syndrome (APS), and a number of other major medical illnesses (Bauer & Lip, 2024). Patients with acquired hypercoagulability have an increased risk of venous thrombosis, arterial thrombosis, or both; however, there is a low risk of recurrence, regardless of thrombophilia status (Connors, 2017). A rare complication of warfarin treatment, warfarin-induced skin necrosis is commonly due to protein C deficiency, with rare cases of protein S deficiency or PVL having been reported (Bauer & Lip, 2023).

Risk factors for arterial thrombosis are lesser known. The relationship between FVL and arterial thrombosis is controversial with studies reporting varying results; overall, FVL is not currently considered a major risk factor for arterial thrombosis (Carroll & Piazza, 2018; Kujovich, 2011). Kujovich (2018) states

that FVL testing should not be performed on persons with any type of arterial thrombosis including myocardial infarction and stroke in children or adults. It has also been reported that while inherited antithrombin, protein S and protein C deficiencies are important risk factors for venous thrombosis, "they have little or no effect on arterial thrombosis" (Previtali et al., 2011). Further, prothrombin gene mutation is not consistently shown to increase the risk of an arterial thromboembolism, and "There is no association of antithrombin deficiency with arterial thrombosis" (Carroll & Piazza, 2018).

It has been proposed that venous thrombosis risk testing may be beneficial as a pre-transplant evaluation test. However, no studies have been identified suggesting this. The North American Thrombosis Forum (NAFT) states that even though certain genetic conditions predispose a small proportion of the population to the development of blood clots, "few people with thrombophilias develop symptoms"; further, there is no cost-effective, safe or long-term method to prevent a blood clot from forming even if a genetic predisposition is identified (NATF, 2019).

Thrombotic events such as thrombophilia and stroke have become increasingly documented in hospitalized pediatric patients with underlying medical conditions such as prematurity, cancer, and congenital heart disease, but they are rarely identified in healthy children. Furthermore, in most cases of pediatric venous thromboembolism, there exist other underlying risk factors such as indwelling central venous catheter and inherited thrombophilia that are worthy of further investigation. The incidence of venous thromboembolisms is highest in neonates and infants, but there is a second peak recorded in adolescence, coinciding with the use of oral contraceptives. However, as in the case with adults, little to no evidence suggests that the use of venous thrombosis risk testing in children will affect the acute management of venous thromboembolisms. In a study including a total of 271 children with VTE, it was found that the relative frequencies of individually inherited thrombophilias were low—for example, the highest recorded frequency of IT disorders was of Factor V Leiden, occurring in only five to 10 percent of the samples. Moreover, a study of 52 children with thromboembolic events during the acute phase did not urge any changes to acute management, regardless of the result of the test (Raffini et al., 2023).

Venous thrombosis risk testing has also been entertained as a manner of combatting pediatric stroke, which can be characterized in a variety of ways, such as by age and by presentation. Arterial ischemic infarctions are the most common, comprising approximately 80% of all perinatal strokes, and this form of stroke can occur in up to one in 3500 of newborns. However, though it would seem reasonable for venous thrombosis risk testing to be employed here, recent prospective case-control studies suggest that routine thrombophilia testing is not warranted. The study showed that conditions associated with thrombophilia rarely coincided with arterial ischemic strokes, and these conditions included, but were not limited to, decreased levels of protein C, protein S, or prothrombin, and genotyping of factor V Leiden (FVL) and factor II (FII, prothrombin) G20210A. Of the 14 parameters examined, 12 showed no difference, including all common thrombophilias examined, with specific mention that FVL and FII were comparable to population norms (Curtis et al., 2017; Ferriero et al., 2019). Subsequent evaluation deemed thrombophilia evaluation in neonates as having limited clinical utility because "levels of protein C, protein S, antithrombin, and factor XI are normally decreased to 30% of adults levels, and these levels only approach adult levels at various time points during childhood". Therefore, the use of thrombophilia testing for these proteins may be misleading in the neonatal period, and MRIs instead should be used to diagnose the thrombosis (Ferriero et al., 2019). Moreover, studies focusing on the roles of thrombophilia, arteriopathy, and cardiac abnormalities in perinatal ischemic stroke find that these risk factors were at best unclear, weakening what predictive power they were believed to contain for even recurrent events after perinatal stroke and leading researchers to conclude that thrombophilia evaluation should rarely be considered in cases of perinatal stroke (Lehman et al., 2017).

While the initial aPTT assays used unaltered plasma (first-generation assays), some versions were neither sensitive nor specific for FVL. Modifications to this test resulted in second generation functional aPC resistance assays that correlate well with the presence of FVL. However, in rare cases, functional assays for aPC resistance can give misleading results (e.g., the presence of a lupis anticoagulant can cause falsely abnormal results in some assays; therapy with a direct thrombin inhibitor or oral factor Za inhibitor can cause falsely normal results). In addition, while FVL can be detected by genetic testing or a second-generation functional coagulation test for aPC resistance, individuals with a positive aPC resistance assay would still need to receive genetic testing to confirm a diagnosis (Bauer, 2023a). Due to difficulty with interpretation, a need for confirmatory genetic testing, and the overall declining cost of genetic testing, aPC resistance assays are performed infrequently. When performed, they are simply reported as positive, borderline, or negative (Middeldrop, 2023).

The term FVL paradox describes the different risk of DVT and PE in FVL carriers; there is data to suggest that FLV carriers are less likely to experience isolated PE (without DVT) than the general population (Bauer, 2023a). de Moerloose et al. (2000) studied the prevalence of FVL in people suspected of DVT and/or PE. The 99 participants with PE were categorized based on "those with PE but without DVT (n = 57) and those with PE and DVT (n = 42)." The odds ratio for the prevalence of FVL was higher (19.1%) in patients with DVT and PE than the odds ratio for the prevalence of FVL in patients with only PE (10.5%), suggesting that "patients with primary PE are less often affected by the factor V Leiden mutation." In another study by (Mäkelburg et al., 2010), the relative risk of DVT in FVL carriers compared to non-carriers was 7.0, while the relative risk of PE in FVL carriers compared to non-carriers was 2.8.

Analytical Validity

Murphy and Sabath (2019) have compared the accuracy and reliability of two tests: a genotypic assay which identifies FVL mutations, and a phenotypic aPC resistance assay. Data from 1596 patients was analyzed; each patient had received both types of testing. The authors state that the phenotypic testing exhibited both high sensitivity and specificity compared to genotypic testing. "Phenotypic assays had close to total concordance with genotypic assays over 16 years of testing. Changing ordering practices could result in up to an 80% reduction in testing costs" (Murphy & Sabath, 2019).

A systematic review and meta-analysis by Chiasakul et al. (2019) researched the relationship between inherited thrombophilia and the risk of arterial ischemic stroke in adults. Inherited thrombophilias included FVL, protein C and S deficiency, antithrombin deficiency and prothrombin G20210A mutation. For this study, 11,916 stroke patients and 96,057 controls were identified. The authors concluded that "Compared with controls, patients with arterial ischemic stroke were significantly more likely to have the following inherited thrombophilias: factor V Leiden (OR, 1.25; 95% CI, 1.08-1.44; I2=0%), prothrombin G20210A mutation (OR, 1.48; 95% CI, 1.22-1.80; I2=0%), protein C deficiency (OR, 2.13; 95% CI, 1.16-3.90; I2=0%), and protein S deficiency (OR, 2.26; 95% CI, 1.34-3.80; I2=8.8%)" (Chiasakul et al., 2019). Antithrombin deficiency did not reach statistical significance in this study. Hence, in this review, inherited thrombophilias were found to be associated with an increased risk of arterial ischemic stroke in adults.

In a systematic review, Ortega studied the predictive value of D-dimer testing for venous thrombosis diagnosis in unusual locations. 3378 patients from 23 articles with thrombosis in unusual sites, such as upper extremity deep vein thrombosis (DVT), cerebral vein thrombosis (CVT) and splanchnic vein thrombosis (SVT), were studied. 12 articles on CVT concluded that timing of D-dimer testing is important and patients with short duration of symptoms displayed higher D-dimer levels. Sensitivity and specificity in these patients ranged from 58% to 97% and from 77% to 97.5%, respectively. The authors conclude that "D-dimer testing should not be currently recommended for the diagnosis of thrombosis in unusual

sites as a first line diagnostic tool. The development of algorithms combining biomarkers such as D-dimer and clinical decision tools could improve the diagnosis" (Ordieres-Ortega et al., 2020).

Clinical Utility and Validity

A D-dimer assay is a blood test that is used in clinical practice to assist in identifying if a patient has a DVT or PE; this test may also help patients experiencing unprovoked VTE to determine if anticoagulation treatment should continue or halt after initial treatment is complete (Linkins & Takach Lapner, 2017). A D-dimer assay may vary greatly based on the type of antibody used, the method of capture, calibration, and instrumentation. Currently, 30 different assays as available which use 20 different monoclonal antibodies; various studies have reported a broad sensitivity and specificity range for D-dimer assays from 69-97% and 43-99% respectively (Linkins & Takach Lapner, 2017). Hence, all D-dimer assays differ and need to be validated within the population of interest. Because of this, comparing study results is challenging.

Factor VIII is a blood clotting protein encoded by the F8 gene. A case report by Algahtani and Stuckey (2019) suggests that high factor VIII levels may also assist in risk factor determination for thrombosis or ischemic heart disease. "We conclude that high factor VIII levels are a risk factor for thrombosis, with a greater impact on venous than on arterial thrombosis. However, due to a lack of international consensus on methods for the laboratory testing of factor VIII levels in plasma, we would not currently recommend the measurement of factor VIII levels as part of routine thrombophilia screening" (Algahtani & Stuckey, 2019). This relationship has been shown previously as elevated levels of coagulation factor VIII:C were identified in a retrospective study of 584 first-degree relatives of 177 patients with high coagulation factor VIII:C levels; the researchers found that 40% of first degree relatives also had high VIII:C levels and were at an increased risk for VTE and arterial thrombosis when compared to other first-degree relatives with normal VIII:C levels (Bank et al., 2005).

Lee et al. (2017) performed whole exome sequencing on 64 patients with VTE to assess the types of mutations of inherited thrombophilias. Of these 64 patients, 39 of them were found to have a pathogenic variant or variant of unknown significance (VUS). Further, eight were found to have a Factor V mutation (six with FVL and two with less common mutations), two were found to have a prothrombin G20210A mutation, six were found to have a protein S mutation, two were found to have a protein C mutation, and three were found to have an antithrombin mutation (Lee et al., 2017).

Segal et al. (2009) reviewed the utility of FVL and prothrombin G20210A testing. The authors reviewed 124 articles and concluded that although genetic testing for these two risk factors is very accurate (valid), the clinical utility is lacking due to lack of evidence demonstrating improvement in clinical outcomes (Segal et al., 2009).

Onda studied the clinical utility of a new diagnostic algorithm based on serum D-dimer levels for VTE after hepatectomy. A total of 742 patients who underwent hepatectomy were enrolled in the study and measured for serum D-dimer levels post-op. CT scan was performed for patients who had a D-dimer level of greater than 20 μg/mL. Based on D-dimer and CT scan, VTE was diagnosed in 26 patients and pulmonary embolism (PE) was diagnosed in 18 patients. Multivariate analysis also showed that a resected liver weight of more than 120 grams is a significant predictor of VTE. Overall, "patients who undergo hepatectomy are at high risk for VTE, especially when the resected liver weight is high. The proposed diagnostic algorithm based on serum D-dimer levels for VTE after hepatectomy can be useful for early diagnosis" (Onda et al., 2021).

Guidelines and Recommendations

American Heart Association/American Stroke Association (AHA/ASA)

The AHA/ASA has issued a scientific statement for the management of stroke in neonates and children, wherein testing for thrombophilic abnormalities are discussed. The AHA/ASA admits that due to the lack of "an adequately powered study to detect the impact of genetic thrombophilia on recurrence risk in pediatric AIS [arterial ischemic stroke], definite recommendations about evaluation remain challenging", but acknowledges that "laboratory testing outside of clinical studies may provide guidance for long-term management of the patient". For cases of thrombophilia the AHA/ASA provides an algorithm for the "Targeted Evaluation of a Child With AIS for Rare Causes or Causes Requiring Additional Evaluation" that includes the examination of Factor VIII level, Lipoprotein(a), *MTHFR* mutation, and homocysteine levels, and it is suggested that "non-DNA testing may need to be repeated when the child is older to ensure that adult levels of proteins have been attained" and "measurement of proteins or homocysteine levels in the acute phase of stroke may not be accurate and should be repeated after the acute event". Finally, for the evaluation of a child with AIS, it is believed that "A thrombophilia evaluation is helpful in every case of childhood stroke, especially if there is no identifiable cause, medical history of thrombosis, or a first-degree relative with thrombosis history" (Ferriero et al., 2019).

In 2021, the AHA released guidelines on stroke prevention. The AHA brushes on testing for hematologic traits in the context of secondary stroke prevention. "If in certain clinical scenarios (eg, paradoxical emboli caused by venous thrombosis or recurrent venous thrombosis) testing for thrombophilic states is considered, testing for protein C, protein S, or antithrombin levels should be deferred or repeated at least 4 to 6 weeks (or up to 6 months for factor VIII609) after the acute stroke given that these protein levels may be altered during the acute stroke phase" (Kleindorfer et al., 2021).

American College of Medical Genetics and Genomics (ACMG)

ACMG has released guidelines for laboratory testing of venous thromboembolism (VTE). This 2018 edition superseded the 2005 edition. The guidelines are as follows:

Testing for factor V Leiden and factor II c.*97G>A (this mutation is also known as G20210A) is recommended in the following circumstances:

- A first unprovoked VTE, especially <50 years old
- VTE at unusual sites (such as hepatic portal, mesenteric, and cerebral veins)
- Recurrent VTE
- Personal history of VTE with (a) two or more family members with a history of VTE or (b) one first-degree relative with VTE at a young age
- Patients with low aPC resistance activity

Testing may be considered in the following circumstances:

- Females under the age of 50 who smoke tobacco and have a history of acute myocardial infarction
- Siblings of individuals known to be homozygous for factor V Leiden or factor II c.*97G>A, because they have a one in four chance of being a homozygote
- Asymptomatic pregnant individual or individual contemplating pregnancy, with a first-degree relative with unprovoked VTE or VTE provoked by pregnancy or contraceptive use

- Pregnant individual or individual contemplating pregnancy or estrogen use who has a first-degree relative with a history of VTE and is a known carrier for factor V Leiden and/or factor II c.97*G>A variant
- Pregnant individual or individual contemplating pregnancy with a previous non-estrogen-related VTE or VTE provoked by a minor risk factor, because knowledge of the factor V Leiden or factor II c.*97G>A status may alter pregnancy related thrombophylaxis (Zhang et al., 2018).

The ACMG found several clinical scenarios requiring special considerations worth mentioning, involving different populations. One involved the testing of asymptomatic versus symptomatic individuals, in which they assert that "It is generally not recommended to test asymptomatic minors as VTE rarely occurs before young adulthood even in the homozygous state." For prenatal testing and population screening, the ACMG suggests that "prenatal testing and population screening are not indicated due to the low penetrance of these variants, later age of onset, and lack of genotype-directed prophylaxis". Lastly, in individuals considering taking estrogen-containing oral contraceptives (OC) or hormone replacement therapy (HRT), the ACMG indicates that "A family and personal history of thrombosis should be carefully evaluated for all women before initiating HRT and a positive history may warrant thrombophilia screening" (Zhang et al., 2018).

ACMG does not support testing for *MTHFR* variants in thrombophilia assessment due to the lack of correlation with negative pregnancy outcomes (Hickey et al., 2013). This statement was reaffirmed in 2020 (Bashford et al., 2020).

American Society of Hematology (ASH)

The 2013 ASH recommends against testing "for thrombophilia in adult patients with venous thromboembolism (VTE) occurring in the setting of major transient risk factors (surgery, trauma or prolonged immobility)" (ASH, 2013).

In 2018, ASH released their guidelines for management of venous thromboembolism, which included the following recommendations (Lim et al., 2018). These guidelines were reviewed in 2022 by an ASH expert working group and agreed to "continue monitoring the supporting evidence rather than revise or retire these guidelines at this time."

- "*Recommends* using a strategy starting with D-dimer for excluding PE in a population with low prevalence/PTP (≤5%), followed by ventilation-perfusion (VQ) scan or computed tomography pulmonary angiography (CTPA) for patients requiring additional testing.
- *Recommends against* using a positive D-dimer alone to diagnose PE, and against additional testing following negative CTPA or normal VQ scan in a population with low prevalence/PTP (≤5%).
- S*uggests* using a strategy starting with D-dimer for excluding PE in a population with intermediate prevalence/PTP (~20%), followed by VQ scan or CTPA for patients requiring additional testing.
- *Recommends against* using a positive D-dimer alone to diagnose PE, and against additional testing following negative CTPA or normal VQ scan in a population with intermediate prevalence/PTP (~20%).
- *Recommends against* using a positive D-dimer alone to diagnose PE, and against using D-dimer as a subsequent test following a negative CT scan in a population with high prevalence/PTP (≥50%).
- *Suggests* using a strategy starting with D-dimer for excluding recurrent PE in a population with unlikely PTP.

- *Recommends* using a strategy starting with D-dimer for excluding DVT in a population with low prevalence/PTP (≤10%), followed by proximal lower extremity ultrasound or whole-leg ultrasound for patients requiring additional testing.
- *Recommends against* using a positive D-dimer alone to diagnose DVT, and against additional testing following negative proximal or whole-leg ultrasound in a population with low prevalence/PTP (≤10%).
- *Recommends against* using a positive D-dimer alone to diagnose DVT in a population with intermediate prevalence/PTP (~25%).
- *Recommends against* using a positive D-dimer alone to diagnose DVT in a population with high prevalence/PTP (≥50%).
- *Suggests* using a strategy starting with D-dimer for excluding recurrent DVT in a population with unlikely PTP.
- *Suggests* a strategy starting with D-dimer for excluding upper extremity DVT in a population with low prevalence/unlikely PTP (10%), followed by duplex ultrasound if D-dimer is positive.
- *Recommends against* using a positive D-dimer alone to diagnose upper extremity DVT in a population with low prevalence/unlikely PTP (10%).
- *Suggests* a strategy of either D-dimer followed by duplex ultrasound/serial duplex ultrasound, or duplex ultrasound/serial duplex ultrasound alone for assessing patients suspected of having upper extremity DVT in a population with high prevalence/likely PTP (40%).
- *Recommends against* using a positive D-dimer alone to diagnose upper extremity DVT in a population with high prevalence/likely PTP (40%)" (Lim et al., 2018).

In 2020, the ASH released guidelines on management of venous thromboembolism. ASH suggest "against the routine use of prognostic scores, D-dimer testing, or venous ultrasound to guide the duration of anticoagulation" (Ortel et al., 2020).

In 2023, the ASH released guidelines thrombophilia testing for the management of venous thromboembolism. "The panel issued a strong recommendation against testing the general population before starting combined oral contraceptives (COCs) and conditional recommendations for thrombophilia testing in the following scenarios: (a) patients with VTE associated with nonsurgical major transient or hormonal risk factors; (b) patients with cerebral or splanchnic venous thrombosis, in settings where anticoagulation would otherwise be discontinued; (c) individuals with a family history of antithrombin, protein C, or protein S deficiency when considering thromboprophylaxis for minor provoking risk factors and for guidance to avoid COCs/hormone replacement therapy; (d) pregnant women with a family history of high-risk thrombophilia types; and (e) patients with cancer at low or intermediate risk of thrombosis and with a family history of VTE. For all other questions, the panel provided conditional recommendations against testing for thrombophilia." The panel also listed the following 23 recommendations (Middeldorp et al., 2023):

- "For patients with unprovoked VTE who have completed primary short-term treatment, the ASH guideline panel suggests not to perform thrombophilia testing to guide the duration of anticoagulant treatment (conditional recommendation based on very low certainty in the evidence about effects)."
- "For patients with VTE provoked by surgery who have completed primary short-term treatment, the ASH guideline panel suggests not to perform thrombophilia testing to determine the duration of anticoagulant treatment (conditional recommendation based on very low certainty in the evidence about effects)."
- "For patients with VTE provoked by a nonsurgical major transient risk factor who have completed primary short-term treatment, the ASH guideline panel suggests testing for thrombophilia to

guide anticoagulant treatment duration. The panel suggests indefinite anticoagulant treatment for patients with thrombophilia and stopping anticoagulant treatment for patients without thrombophilia (conditional recommendation based on very low certainty in the evidence about effects)."

- "For women with VTE provoked by pregnancy or postpartum who have completed primary treatment, the ASH guideline panel suggests thrombophilia testing to guide anticoagulant treatment duration. The panel suggests indefinite anticoagulant treatment for women with thrombophilia and stopping anticoagulant treatment for women without thrombophilia (conditional recommendation based on very low certainty in the evidence about effects)."
- "For women with VTE associated with COCs who have completed primary short-term treatment, the ASH guideline panel suggests testing for thrombophilia to guide anticoagulant treatment duration. The panel suggests indefinite anticoagulant treatment for women with thrombophilia and stopping anticoagulant treatment for women without thrombophilia (conditional recommendation based on very low certainty in the evidence about effects)."
- "For patients with an unspecified type of VTE who have completed primary short-term treatment, the ASH guideline panel suggests not performing thrombophilia testing to guide anticoagulant treatment duration (conditional recommendation based on very low certainty in the evidence about effects)."
- "For patients with CVT who have completed primary treatment in a setting where anticoagulation would be discontinued, the ASH guideline panel suggests thrombophilia testing to guide anticoagulant treatment duration. The panel suggests indefinite anticoagulation for patients with thrombophilia (conditional recommendation based on very low certainty in the evidence about effects)."
- "For patients with CVT who have completed primary treatment in a setting where anticoagulation would be continued indefinitely, the ASH guideline panel suggests not to perform thrombophilia testing to guide anticoagulant treatment duration (conditional recommendation based on very low certainty in the evidence about effects)."
- "For patients with splanchnic venous thrombosis who have completed primary treatment in a setting where anticoagulation would be discontinued, the ASH guideline panel suggests thrombophilia testing to guide anticoagulant treatment duration. The panel suggests indefinite anticoagulation for patients with thrombophilia (conditional recommendation based on very low certainty in the evidence about effects)."
- "For patients with splanchnic venous thrombosis who have completed primary treatment in a setting where anticoagulation would be continued indefinitely, the ASH guideline panel suggests not performing thrombophilia testing to guide anticoagulant treatment duration (conditional recommendation based on very low certainty in the evidence about effects)."
- "For individuals with a family history of VTE and known FVL or PGM [prothrombin 20210A gene mutation] (low-risk thrombophilia) who have a minor provoking risk factor for VTE (eg, immobility or minor injury, illness, or infection), the ASH guideline panel suggests not testing for the known familial thrombophilia to guide thromboprophylaxis (conditional recommendation based on very low certainty in the evidence about effects). For individuals with a family history of VTE and known antithrombin, protein C, or protein S deficiency (high-risk thrombophilia) who have a minor provoking risk factor for VTE, the ASH guideline panel suggests testing for the known familial thrombophilia. The panel suggests thromboprophylaxis in individuals with thrombophilia and no thromboprophylaxis in individuals without thrombophilia (conditional recommendation based on very low certainty in the evidence about effects)."
- "For individuals with a family history of VTE and known FVL or PGM (low-risk thrombophilia) who have a minor provoking risk factor for VTE (eg, immobility or minor injury, illness, or infection), the

ASH guideline panel suggests not testing for all hereditary thrombophilias to guide thromboprophylaxis (conditional recommendation based on very low certainty in the evidence about effects). For individuals with a family history of VTE and known antithrombin, protein C, or protein S deficiency (high-risk thrombophilia) who have a minor provoking risk factor for VTE, the ASH guideline panel suggests testing for all hereditary thrombophilias (using a panel of tests). The panel suggests thromboprophylaxis in individuals with thrombophilia and no thromboprophylaxis for a minor provoking risk factor in individuals without thrombophilia (conditional recommendation based on very low certainty in the evidence about effects)."

- "For individuals with a family history of VTE and unknown thrombophilia status in the family who have a minor provoking risk factor for VTE (eg, immobility or minor injury, illness, or infection), the ASH guideline panel suggests not testing for all hereditary thrombophilias (using a panel of tests) to guide thromboprophylaxis (conditional recommendation based on very low certainty in the evidence about effects)."
- "For individuals with a family history of FVL or PGM (low-risk thrombophilia) but no family history of VTE who have a minor provoking risk factor for VTE (eg, immobility or minor injury, illness, or infection), the ASH guideline panel suggests not testing for the known thrombophilia to guide thromboprophylaxis (conditional recommendation based on very low certainty in the evidence about effects). For individuals with a first-degree family history of antithrombin, protein C, or protein S deficiency (high-risk thrombophilia) but no family history of VTE who have a minor provoking risk factor for VTE, the ASH guideline panel suggests testing for the known thrombophilia. The panel suggests thromboprophylaxis in individuals with thrombophilia and no thromboprophylaxis in individuals without thrombophilia (conditional recommendation based on very low certainty in the evidence about effects). For individuals with a second-degree family history of antithrombin, protein C, or protein S deficiency (high-risk thrombophilia) but no family history of VTE who have a minor provoking risk factor for VTE, the ASH guideline panel suggests either testing for the known thrombophilia or not testing for thrombophilia to guide the use of thromboprophylaxis (conditional recommendation based on very low certainty in the evidence about effects)."
- "For women from the general population who are considering using COCs, the ASH guideline panel recommends not performing thrombophilia testing to guide the use of COC (strong recommendation based on low certainty in the evidence about effects)."
- "For women from the general population who are considering using HRT, the ASH guideline panel suggests not performing thrombophilia testing to guide the use of HRT (conditional recommendation based on low certainty in the evidence about effects)."
- "For women with a family history of VTE and unknown thrombophilia status in the family who are considering using COCs, the ASH guideline panel suggests not testing for hereditary thrombophilia (using a panel of tests) to guide the use of COC (conditional recommendation based on very low certainty in the evidence about effects)."
- "For women with a family history of VTE and unknown thrombophilia in the family who are considering using HRT, the ASH guideline panel suggests not performing thrombophilia testing for any hereditary thrombophilia to guide the use of HRT (conditional recommendation based on very low certainty in the evidence about effects)."
- "For women with a family history of VTE and known FVL or PGM in the family (low-risk thrombophilia), the ASH guideline panel suggests not testing for the known familial thrombophilia to guide the use of COC (conditional recommendation based on very low certainty in the evidence about effects). For women with a family history of VTE and known antithrombin, protein C, or protein S deficiency in the family (high-risk thrombophilia), the ASH guideline panel suggests testing for the known familial thrombophilia. The panel suggests avoidance of COCs for

women with high-risk thrombophilia (conditional recommendation based on very low certainty in the evidence about effects)."

- "For women with a family history of VTE and known FVL or PGM in the family (low-risk thrombophilia), the ASH guideline panel suggests not testing for the known familial thrombophilia to guide the use of HRT (conditional recommendation based on very low certainty in the evidence about effects). For women with a family history of VTE and known antithrombin, protein C, or protein S deficiency in the family (high-risk thrombophilia), the ASH guideline panel suggests testing for the known familial thrombophilia. The panel suggests avoidance of HRT for women with high-risk thrombophilia (conditional recommendation based on very low certainty in the evidence about effects)."
- "For women with a family history of VTE and known homozygous FVL, a combination of FVL and PGM, or an antithrombin deficiency in the family, the ASH guideline panel suggests testing for the known familial thrombophilia. The panel suggests antepartum thromboprophylaxis for women with the same familial thrombophilia (ie, homozygous FVL, combination of FVL and PGM, or antithrombin deficiency) and no antepartum prophylaxis for women without the same familial thrombophilia (conditional recommendation based on very low certainty in the evidence about effects). For women with a family history of VTE and a known protein C or protein S deficiency in the family, the ASH guideline panel suggests either testing for the known familial thrombophilia or not testing for thrombophilia to guide antepartum prophylaxis (conditional recommendation based on very low certainty in the evidence about effects)."
- "For women with a first-degree family history of VTE and known homozygous FVL, a combination of FVL and PGM, antithrombin deficiency, protein C deficiency, or protein S deficiency in the family, the ASH guideline panel suggests testing for the known familial thrombophilia. The panel suggests postpartum thromboprophylaxis for women with the same familial thrombophilia (ie, homozygous FVL, combination of FVL and PGM, or antithrombin deficiency) and no postpartum prophylaxis for women without the same familial thrombophilia (conditional recommendation based on very low certainty in the evidence about effects). For women with a second-degree family history of VTE and a known combination of FVL and PGM, or antithrombin deficiency in the family, the ASH guideline panel suggests testing for the known familial thrombophilia. The panel suggests postpartum thromboprophylaxis for women with thrombophilia and no postpartum prophylaxis for women without thrombophilia (conditional recommendation based on very low certainty in the evidence about effects). For women with a second-degree family history of VTE and a known protein C or protein S deficiency in the family, the ASH guideline panel suggests either testing for the known familial thrombophilia or not testing for thrombophilia to guide postpartum thromboprophylaxis (conditional recommendation based on very low certainty in the evidence about effects)."
- "For ambulatory patients with cancer receiving systemic therapy who have a family history of VTE and are otherwise determined to be at low or intermediate risk for VTE, the ASH guideline panel suggests testing for hereditary thrombophilia. The panel suggests ambulatory thromboprophylaxis for patients with thrombophilia and no thromboprophylaxis for patients without thrombophilia (conditional recommendation based on very low certainty in the evidence about effects)."

American College of Obstetricians and Gynecologists

The 2013 ACOG clinical management guidelines recommend that screening for inherited thrombophilia "may be considered in the following clinical settings:

1. "A personal history of venous thromboembolism that was associated with a non-recurrent risk

factor
2. A first degree relative (parent or sibling) with a history of high-risk thrombophilia" (ACOG, 2013).

The 2018 ACOG Practice Bulletin Summary Number 197 supersedes the above 2013 guidelines (Practice Bulletin Number 138). In this update, the ACOG makes the following recommendations regarding screening based on "limited or inconsistent scientific evidence":

"Screening for inherited thrombophilias is not recommended for women with a history of fetal loss or adverse pregnancy outcomes including abruption, preeclampsia, or fetal growth restriction because there is insufficient clinical evidence that antepartum prophylaxis with unfractionated heparin or low molecular-weight heparin prevents recurrence in these patients."

"Because of the lack of association between either heterozygosity or homozygosity for the *MTHFR* C677T polymorphism and any negative pregnancy outcomes, including any increased risk of VTE, screening with either *MTHFR* mutation analyses or fasting homocysteine levels is not recommended."

The 2018 ACOG recommends the following screening guideline based on "consensus and expert opinion":

"Among women with personal histories of VTE, recommended screening tests for inherited thrombophilias should include factor V Leiden mutation; prothrombin G20210A mutation; and antithrombin, protein S, and protein C deficiencies" (ACOG, 2018).

Evaluation of Genomic Applications in Practice and Prevention

"The Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Working Group found adequate evidence to recommend against *routine* testing for Factor V Leiden (FVL) and/or prothrombin 20210G>A (Gupta et al.) in the following circumstances: (1) adults with idiopathic venous thromboembolism (VTE). In such cases, longer term secondary prophylaxis to avoid recurrence offers similar benefits to patients with and without one or more of these mutations. (2) Asymptomatic adult family members of patients with VTE and an FVL or PT mutation, for the purpose of considering primary prophylactic anticoagulation. Potential benefits are unlikely to exceed potential harms. The evidence was insufficient to determine whether FVL/PT testing might have clinical utility in some circumstances, such as for identifying FVL homozygosity among asymptomatic family members of adults with idiopathic VTE or counseling patients about the risks and benefits of antithrombotic therapy. The recommendations do not extend to patients with other risk factors for thrombosis, such as contraceptive use, as the evidence review that serves as the basis for the recommendations focused primarily on idiopathic VTE" (EGAPP, 2011).

The Anticoagulation Forum

The Anticoagulation Forum published guidance in the Journal of Thrombosis and Thrombolysis on (Stevens et al., 2016):

- "Do not perform thrombophilia testing following an episode of provoked VTE. A positive thrombophilia evaluation is not a sufficient basis to offer extended anticoagulation following an episode of provoked VTE.
- Do not perform thrombophilia testing in patients following an episode of unprovoked VTE. If a patient with unprovoked VTE and low bleeding risk is planning to stop anticoagulation, test for thrombophilia if test results would change this decision. A negative thrombophilia evaluation is

not a sufficient basis to stop anticoagulants following an episode of unprovoked VTE in a patient with low bleeding risk and willingness to continue therapy.
Heterozygosity for FVL or PGM does not increase the predicted risk of recurrence after unprovoked VTE to a clinically significant degree.

- Do not test for thrombophilia in asymptomatic family members of patients with VTE or hereditary thrombophilia. As a family history of VTE confers an excess risk of thrombosis, relatives should be counseled regarding use of prophylaxis in high-risk situations.
- Do not test for thrombophilia in asymptomatic family members of patients with VTE or hereditary thrombophilia who are contemplating use of estrogen. If an [individual] is contemplating estrogen use has a first-degree relative with VTE and a known hereditary thrombophilia, test for that thrombophilia if the result would change the decision to use estrogen.
- Do not perform thrombophilia testing at the time of VTE diagnosis or during the initial 3-month course of anticoagulant therapy. When testing for thrombophilias following VTE, use either a 2-stage testing approach or perform testing after a minimum of 3 months of anticoagulant therapy has been completed, and anticoagulants have been held.
- Do not test for thrombophilia in asymptomatic family members of patients with VTE or hereditary thrombophilia who are contemplating pregnancy. If [an individual] contemplating pregnancy has a first-degree relative with VTE and a known hereditary thrombophilia… test for that thrombophilia if the result would change VTE prophylaxis decisions" (Stevens et al., 2016).

American College of Cardiology (ACC)

In 2017, guidance published in the New England Journal of Medicine by Gupta was summarized by Barnes for the American College of Cardiology:

1. "Venous thromboembolism (VTE) affects an estimated 300,000-600,000 patients annually in the United States.
2. The risk of VTE recurrence is best predicted by whether the initial VTE episode was provoked or unprovoked, not the results of inherited thrombophilia testing.
3. Most patients with a provoked VTE have recently undergone surgery, immobility, trauma, or have a concurrent cancer diagnosis. Concurrent use of hormones (e.g., estrogen-containing contraceptive pills) is also frequently considered a provoking factor for VTE development.
4. For patients with a first provoked VTE event, guidelines recommend anticoagulation for only 3 months (not longer). Prolonged anticoagulation is associated with an increased risk of bleeding that outweighs the risk of VTE recurrence for these patients.
5. Patients with an unprovoked VTE (none of the provoking risk factors listed above) require longer anticoagulation due to a higher risk of recurrence that outweighs the risk of bleeding associated with long-term anticoagulation therapy.
6. Thrombophilia testing performed in the setting of an acute clot or ongoing anticoagulation therapy will often result in spurious results (usually false positive). For example, natural anticoagulants (e.g., protein C and S, antithrombin) are consumed during an acute thrombotic event and the levels can be reduced by ongoing anticoagulant therapy.
7. A recent study identified that up to 55% of Medicare patients with provoked VTE had undergone inappropriate thrombophilia testing, associated with significant cost to the healthcare system.
8. While thrombophilia testing rarely impacts management decisions about anticoagulation therapy, it may be beneficial for genetic testing purposes in patients presenting with a first unprovoked VTE at a young age (e.g., <45 years) or at an unusual site.
9. For patients with unprovoked VTE at a young age, VTE at an unusual site, arterial thrombosis, or pregnancy morbidity, testing for antiphospholipid antibodies, JAK2 mutation, and paroxysmal

nocturnal hemoglobinuria may be beneficial.
10. There is no role for extensive cancer screening (e.g., computed tomography scanning) in patients with VTE. Only routine, age-appropriate cancer screening is recommended" (G. Barnes, 2017; Gupta et al., 2017).

Again in 2017, key points—inclusive of guiding points—published in the New England Journal of Medicine by Connors were captured by Barnes for the American College of Cardiology:

1. "The majority of patients with venous thromboembolism (VTE) should not be tested for thrombophilia. Data supporting clinical usefulness and benefits are limited or nonexistent.
2. Most patients with inherited thrombophilia can be identified by coagulation experts based on the patient's personal and family history of VTE. Thrombophilia testing is usually not required.
3. Factors associated with an inherited thrombophilia include VTE at a young age (<40-50 years), a strong family history of VTE, VTE in conjunction with weak provoking factors at a young age, recurrent VTE, and VTE in an unusual site (e.g., cerebral or splanchnic veins).
4. Do not perform thrombophilia testing at the time of a VTE event, as it can be inaccurate (often false positive). Perform testing (when indicated) after completion of initial therapy and if it might change management strategies.
5. Do not perform thrombophilia testing while a patient is receiving anticoagulation. Instead, wait until 2 weeks after discontinuing warfarin, or 2 days for direct oral anticoagulants and heparin.
6. The goal of thrombophilia testing should be to aid decision making regarding future VTE prophylaxis, to guide testing of family members, and to determine the cause in severe or fatal VTE. Test results alone should not be used to decide on the duration of anticoagulation therapy.
7. Most VTE recurrence risk tools do not incorporate thrombophilia test results into their risk stratification schemes.
8. For patients with provoked VTE, even if they have homozygous factor V Leiden, prothrombin gene mutations, or deficiencies of protein S, C, or antithrombin, they do not require lifelong anticoagulation.
9. Currently available thrombophilia tests are insufficient to identify inherited risks of VTE. Therefore, a negative test should not be interpreted as a patient being free of thrombophilia.
10. Testing for the antiphospholipid antibody syndrome may be useful in patients with unprovoked VTE if there is clinical equipoise about extended anticoagulation courses. It can also be useful to determine warfarin versus direct oral anticoagulant therapy" (G. D. Barnes, 2017; Connors, 2017).

European Society of Cardiology

The ESC has published guidelines for the diagnosis and management of acute PE. These guidelines state:

- "D-dimer measurement and clinical prediction rules should be considered to rule out PE during pregnancy or the post-partum period
- Plasma D-dimer measurement, preferably using a highly sensitive assay, is recommended in outpatients/emergency department patients with low or intermediate clinical probability, or those that are PE-unlikely, to reduce the need for unnecessary imaging and irradiation
- A D-dimer test, using an age-adjusted cut-off or adapted to clinical probability, should be considered as an alternative to the fixed cut-off level
- D-dimer measurement is not recommended in patients with high clinical probability, as a normal result does not safely exclude PE, even when using a highly sensitive assay
- Assessment of the RV [right ventricle] by imaging methods or laboratory biomarkers should be considered, even in the presence of a low PESI [Pulmonary Embolism Severity Index] or a negative

sPESI [simplified Pulmonary Embolism Severity Index]" (Konstantinides et al., 2019).

In 2021, the ESC Working Group released guidelines on diagnosis and management of acute deep vein thrombosis. These guidelines suggest that "ELISA D-dimer or highly sensitive immunoturbidimetric tests should be measured in 'unlikely' clinical probability patients to exclude DVT diagnosis" (Mazzolai et al., 2022).

World Health Organization (WHO)

In the WHO medical eligibility criteria for contraceptive use, known thrombogenic mutations (e.g. factor V Leiden; prothrombin mutation; protein S, protein C, and antithrombin deficiencies) are "a condition which represents an unacceptable health risk if the contraceptive method is used" when considering combined hormonal contraceptives, and the method is "not to be used." The WHO notes that "among women with thrombogenic mutations, COC users had a 2- to 20-fold higher risk of thrombosis than non-users." When considering progestogen-only contraceptives, intrauterine devices, barrier methods, or female surgical sterilization, known thrombogenic mutations are "a condition for which there is no restriction for the use of the contraceptive method" or "the advantages of using the method generally outweigh the theoretical or proven risks." In all cases, the WHO clarifies that "routine screening is not appropriate because of the rarity of the conditions and the high cost of screening" (WHO, 2015).

National Institute for Health and Care Excellence (NICE)

In the NICE venous thromboembolic disease guidelines, for thrombophilia testing, it is recommended:

- "Do not offer testing for hereditary thrombophilia to people who are continuing anticoagulation treatment.
- "Do not offer thrombophilia testing to people who have had provoked DVT or PE."
- "Consider testing for antiphospholipid antibodies in people who have had unprovoked DVT or PE if it is planned to stop anticoagulation treatment, but be aware that these tests can be affected by anticoagulants and specialist advice may be needed."
- "Consider testing for hereditary thrombophilia in people who have had unprovoked DVT or PE and who have a first-degree relative who has had DVT or PE if it is planned to stop anticoagulation treatment, but be aware that these tests can be affected by anticoagulants and specialist advice may be needed."
- "Do not routinely offer thrombophilia testing to first-degree relatives of people with a history of DVT or PE and thrombophilia" (NICE, 2023).

American College of Radiology (ACR)

The ACR has guidelines for suspected pulmonary embolisms (PE). The ARC prefaced that their publication "focuses on the initial evaluation for clinically suspected PE, recognizing that as many as 80% of PE cases are associated with DVT" and that "PE also may occur without detectable DVT." The guidelines do not mention any laboratory or genetic testing, although some of the imaging recommendations are based on positive or negative D-dimer results (Kirsch et al., 2022).

Thrombosis Canada

Thrombosis Canada released guidelines on the diagnosis of PE (Thrombosis Canada, 2023). They state: "The constellation of symptoms and signs may be suggestive of PE but do not alone have the sensitivity or specificity to rule in or rule out the diagnosis. When the diagnosis of PE is considered, the clinical

stability of the patient and associated pre-test probability will dictate the diagnostic approach." The guidelines further recommend:

- "In patients without hypotension (SBP >90 mmHg), pre-test probability can be assessed by a validated clinical prediction rule such as the Well's score. In patients who are <50 years of age, with a low pretest probability of PE, further testing (such as d-dimer measurement or diagnostic imaging) is not necessary provided all clinical features/criteria in the Pulmonary Embolism Rule-out Criteria (PERC) are present."
- "In cases with PE unlikely pre-test probability, a negative high sensitivity D-dimer result rules out the diagnosis of PE. However, a positive D-dimer test MUST be followed up with a definitive test to confirm/refute the diagnosis of PE.
- When a high-sensitive d-dimer assay is used, age-adjusted D-dimer levels can increase the specificity of D-dimer testing without sacrificing sensitivity. In patients over the age of 50, a D-dimer result is considered negative if it is less than the patient age multiplied by 10 (for example, in a 76-year-old, a negative result is less than 760 µg/L). For patients under the age of 50, a D-dimer value less than 500 µg/L remains the cutoff for a negative result.
- With a high PE pre-test probability (Well's score > 4.5), there is no role for ordering a D-dimer, as the clinical likelihood of PE remains unacceptably high among those with a negative D-dimer result. Therefore, when the Wells score is 4.5 or greater, one should go directly to imaging to establish the diagnosis" (Thrombosis Canada, 2023).

The Wells Score is a total score of: clinical symptoms and signs of DVT (3 points), previous DVT or PE (1.5 points), immobilization of over three days or surgery within four weeks (1.5 points), heart rate over 100 beats per minute (1.5 points), hemoptysis (1 point), malignancy (1 point), and no alternative diagnosis more likely than PE (3 points). A total score under 4.5 equates to PE unlikely, and a total score of 4.5 or over equates to PE likely (Thrombosis Canada, 2023).

The PE Rule-out Criteria (PERC) for patients with low pretest probability for PE are: age less than 50, initial heart rate of less than 100 beats per minute, initial SaO2 of over 94% on room air, no unilateral leg swelling, no hemoptysis, no surgery or trauma in the past four weeks, no history of VTE and no estrogen use (Thrombosis Canada, 2023).

Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, please visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
85300	Clotting inhibitors or anticoagulants; antithrombin III, activity
85301	Clotting inhibitors or anticoagulants; antithrombin III, antigen assay
85302	Clotting inhibitors or anticoagulants; protein C, antigen
85303	Clotting inhibitors or anticoagulants; protein C, activity
85305	Clotting inhibitors or anticoagulants; protein S, total
85306	Clotting inhibitors or anticoagulants; protein S, free
85307	Activated Protein C (APC) resistance assay

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

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Revision History

Revision Date	Summary of Changes
09/04/2024	Reviewed and Updated: Updated the background, guidelines and recommendations, and evidence-based scientific references. Literature review necessitated the following changes in coverage criteria: Added "or pulmonary embolism (PE)." to CC1a Corrected reference to Note 3 in CC1.e.

Vitamin B12 and Methylmalonic Acid Testing

Policy Number: AHS – G2014 – Vitamin B12 and Methylmalonic Acid Testing	Initial Presentation Date: 11/16/2015 Effective Date: 4/1/2025

Policy Description

Vitamin B12, also known as cobalamin, is a water-soluble vitamin required for proper red blood cell formation, key metabolic processes, neurological function, and DNA regulation and synthesis. Hematologic and neuropsychiatric disorders caused by a deficiency in B12 can often be reversed by early diagnosis and prompt treatment (Oh & Brown, 2003).

Methylmalonic acid (MMA) is produced from excess methylmalonyl-CoA that accumulates when Vitamin B12 is unavailable and is considered an indicator of functional B12 deficiency (Sobczynska-Malefora et al., 2014).

Holotranscobalamin (holoTC) is the metabolically active fraction of B12 and is an emerging marker of impaired vitamin B12 status (Langan & Goodbred, 2017).

Related Policies

Policy Number	Policy Title
AHS-G2050	Cardiovascular Disease Risk Assessment
AHS-G2154	Folate Testing
AHS-M2141	Testing of Homocysteine Metabolism-Related Conditions

Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) Total vitamin B12 (serum cobalamin) testing **MEETS COVERAGE CRITERIA** once every three months for **any** of the following situations:
    a) For individuals with the following signs and symptoms of vitamin B12 deficiency:
        i) Cutaneous
            (a) Hyperpigmentation
            (b) Jaundice
            (c) Vitiligo
        ii) Gastrointestinal
            (a) Glossitis
        iii) Hematologic
            (a) Anemia (macrocytic, megaloblastic)
            (b) Leukopenia
            (c) Pancytopenia
            (d) Thrombocytopenia
            (e) Thrombocytosis
        iv) Neuropsychiatric
            (a) Areflexia
            (b) Cognitive impairment (including dementia-like symptoms and acute psychosis)
            (c) Gait abnormalities
            (d) Irritability
            (e) Loss of proprioception and vibratory sense
            (f) Olfactory impairment
            (g) Peripheral neuropathy
    b) For individuals undergoing treatment for vitamin B12 deficiency.
    c) For individuals with one or more of the following risk factors for vitamin B12 deficiency:
        i) For individuals with decreased ileal absorption due to:
            (a) Crohn's disease.
            (b) Ileal resection.
            (c) Tapeworm infection.

(d) Having undergone, or for those who have been scheduled for, bariatric procedures such as Roux-en-Y gastric bypass, sleeve gastrectomy, or biliopancreatic diversion/duodenal switch.

ii) For individuals with decreased intrinsic factor due to:

(a) Atrophic gastritis.

(b) Pernicious anemia.

(c) Postgastrectomy syndrome.

iii) For individuals with transcobalamin II deficiency.

iv) For individuals with inadequate B12 intake:

(a) Due to alcohol abuse.

(b) In individuals older than 75 years or elderly individuals being evaluated for dementia.

(c) In vegans or strict vegetarians (including exclusively breastfed infants of vegetarian/vegan mothers).

(d) Due to an eating disorder.

v) For individuals with prolonged medication use:

(a) Histamine H2 blocker use for more than 12 months.

(b) Metformin use for more than four months.

(c) Proton pump inhibitor use for more than 12 months.

2) In asymptomatic high-risk individuals with low-normal levels of vitamin B12 or when vitamin B12 deficiency is suspected but the serum vitamin B12 level is normal or low-normal, methylmalonic acid testing to confirm vitamin B12 deficiency **MEETS COVERAGE CRITERIA**.

3) For the evaluation of inborn errors of metabolism, methylmalonic acid testing **MEETS COVERAGE CRITERIA**.

4) In healthy, asymptomatic individuals, screening for vitamin B12 deficiency **DOES NOT MEET COVERAGE CRITERIA.**

5) For the confirmation of vitamin B12 deficiency, homocysteine testing **DOES NOT MEET COVERAGE CRITERIA**.

6) For the screening, testing, or confirmation of vitamin B12 deficiency, holotranscobalamin testing **DOES NOT MEET COVERAGE CRITERIA**.

7) For all other situations not described above, total vitamin B12 (serum cobalamin) testing **DOES NOT MEET COVERAGE CRITERIA.**

Table of Terminology

Term	Definition
AACE	American Association of Clinical Endocrinology
AAFP	American Academy of Family Physicians

ACE	American College of Endocrinology
ACG	American College of Gastroenterology
ADA	American Diabetes Association
APA	American Psychiatric Association
ASMBS	American Society for Metabolic and Bariatric Surgery
ATT	Anti-tuberculosis treatment
BCMA	British Columbia Medical Association
BMJ	British Medical Journal
BOMSS	British Obesity and Metabolic Surgery Society
BPD/DS	Biliopancreatic diversion/duodenal switch
CBL	Cobalamin
CD	Celiac disease
CDC	Centers for Disease Control and Prevention
CLIA ’88	Clinical Laboratory Improvement Amendments of 1988
CMS	Centers for Medicare and Medicaid
CoA	Coenzyme A
CVD	Cardiovascular disease
DNA	Deoxyribonucleic acid
E-HOD	European network and registry for homocystinurias and methylation defects
EL	Evidence level
FDA	Food and Drug Administration
GPP	Good practice point
H2	Histamine receptor H2
HAART	Highly active antiretroviral therapy
HCY	Homocysteine
HIV	Human immunodeficiency virus
holoTC	Holotranscobalamin
HQO	Health Quality Ontario
HR	Hazard ratio
LDT	Laboratory-developed test
LSG	Laparoscopic sleeve gastrectomy
MBS	Metabolic and bariatric surgery
MMA	Methylmalonic Acid
MTHFR	Methylenetetrahydrofolate reductase
NHANES	National Health and Nutrition Examination Survey
NICE	National Institute for Health and Care Excellence
OMA	Obesity Medicine Association
RYGB	Roux-en-Y gastric bypass
SG	Sleeve gastrectomy
TB	Tuberculosis
tHCy	Total homocysteine
TIBC	Total iron-binding capacity
TOS	The Obesity Society

USPSTF	United States Preventative Services Task Force
WLS	Weight loss surgical

Scientific Background

Vitamin B12 cannot be synthesized by human cells (Means Jr & Fairfield, 2023); rather, it is obtained from animal-derived dietary sources, such as meat, eggs, and dairy products (Hunt et al., 2014), as well as fortified cereals and supplements (Zeuschner et al., 2013). Vitamin B12 deficiency is classically caused by pernicious anemia; however, with modern fortification of western diets, this condition now accounts for only a minority of cases and currently occurs most often due to malabsorption (Means Jr & Fairfield, 2023).

The prevalence of vitamin B12 deficiency in the United States and United Kingdom is approximately 6% in persons younger than 60 years, reaching 20% in those older than 60 years. On the contrary, the prevalence is approximately 40% in Latin America, 70% in Kenyan school children, 80% in East Indian preschool-aged children, and 70% in East Indian adults (Hunt et al., 2014). Risk factors for deficiency include: decreased ileal absorption (Crohn disease, ileal resection, tapeworm infection), decreased intrinsic factor (atrophic gastritis, pernicious anemia, post-gastrectomy syndrome), genetic defects (transcobalamin II deficiency), inadequate intake (alcohol abuse, patients older than 75 years, vegans, or strict vegetarians), prolonged medication use (histamine H2 blocker use for more than 12 months, metformin use for more than four months, proton pump inhibitor use for more than 12 months) (Langan & Goodbred, 2017).

Vitamin B12 plays an essential role in nucleic acid synthesis. Deficiency can result in cell cycle arrest in the S phase or cause apoptosis (Green, 2017) and ultimately bone marrow failure and demyelinating nervous system disease (Stabler, 2013). Vitamin B12 is also critical in the remethylation of homocysteine (Hcy), and deficiency in Vitamin B12 can lead to hyperhomocysteinemia, a condition that has been associated with various cancers, such as breast and ovarian cancers, as well as Parkinson disease (Fan et al., 2020; Hama et al., 2020).

Clinical manifestations of Vitamin B12 deficiency vary in their presence and severity from mild fatigue to severe neurologic impairment (Langan & Goodbred, 2017). Mild deficiency can present as fatigue and anemia with an absence of neurological features. Moderate deficiency may include obvious macrocytic anemia with some mild or subtle neurological features. Severe deficiency shows evidence of bone marrow suppression, clear evidence of neurological features, and risk of cardiomyopathy. Recent literature also suggests a relationship between Vitamin B12 and depression (Sangle et al., 2020).

Vitamin B12 deficiency can cause glossitis and other gastrointestinal symptoms that vary with underlying diseases, such as inflammatory bowel disease or celiac disease (Means Jr & Fairfield, 2023). Early detection and correction of vitamin B12 deficiency with supplementation prevents progression to macrocytic anemia, elevated homocysteine (Hcy), potentially irreversible peripheral neuropathy, memory loss, and other cognitive deficits (Sobczynska-Malefora et al., 2014).

Analytical Validity

Both the clinical recognition of vitamin B12 deficiency and confirmation of the diagnosis by means of testing can be difficult. Several laboratory measures reflecting physiological, static, and functional B12 status have been developed (Hunt et al., 2014); however, there is no universally agreed upon gold standard assay for determining cobalamin levels in humans. The current convention is to estimate the

abundance of vitamin B12 using total serum vitamin B12, despite the low sensitivity of this test (Sobczynska-Malefora et al., 2014). Two reportedly highly sensitive vitamin B12 deficiency markers are elevated levels of serum homocysteine and methylmalonic acid, but testing is expensive, and many other conditions may cause an elevation in these markers, including familial hyperhomocysteinemia, folate deficiency, levodopa therapy, and renal insufficiency (Langan & Zawistoski, 2011). Serum methylmalonic acid levels tend to be just as sensitive but more specific than serum homocysteine levels in regards to vitamin B12 deficiency testing, highlighting the former as the preferred testing method by many (Langan & Zawistoski, 2011).

An in-depth meta-analysis by Willis et al. (2011) of serum cobalamin testing included data from 54 different studies. The variability for sensitivity and specificity across the different studies ranged from 13% to 75% for sensitivity and 45% to 100% for specificity, depending on the reference standard used. Researchers conclude that "from the available evidence, diagnosis of conditions amenable to cbl [vitamin B12] supplementation on the basis of cbl [vitamin B12] level alone cannot be considered a reliable approach to investigating suspected vitamin deficiency" (Willis et al., 2011). The test measures total serum cobalamin including both serum holohaptocorrin and serum holotranscobalamin, which may mask true deficiency or falsely imply a deficient state (Hunt et al., 2014).

Vitamin B12 deficiency is present in both infant and pregnant individuals, and monitoring vitamin B12 levels is important in determining maternal and fetal health and growth. Low vitamin B12 levels during pregnancy are associated with a greater risk of preterm birth (Rogne et al., 2017). It seems that current pregnancy-specific cutoffs for vitamin B12 biomarkers are inadequate in the medical field (Schroder et al., 2019). Recently, a new study has identified a novel cutoff value in the vitamin B12 serum of newborns; the B12-related metabolite known as homocysteine (Hcy) is now recommended to have a cutoff value at "4.77 μmol/L (68.4% sensitivity, 58.3% specificity, p = .012) for the detection of vit-B12 deficiency" (Yetim et al., 2019). Other pregnancy specific B12 biomarkers have been published. According to another study, "The central 95% reference interval limits indicated that serum total B-12 <89.9 and <84.0 pmol/L, holoTC <29.5 and <26.0 pmol/L and MMA >371 and >374 nmol/L, in the first and second trimesters, respectively, may indicate B-12 deficiency in pregnant [individuals]. The lower limits of total B-12 and holoTC and the upper limits of MMA significantly differed by ethnicity in both trimesters. According to the change point analysis, total B-12 <186 and <180 pmol/L and holoTC <62.2 and <67.5 pmol/L in the first and second trimesters, respectively, suggested an increased probability of impaired intracellular B-12 status, with no difference between ethnicities" (Schroder et al., 2019).

Elevated levels of downstream metabolites, MMA and Hcy, are commonly used as adjuvant diagnostics to confirm a suspected diagnosis of cobalamin deficiency (Berg & Shaw, 2013). The sensitivity of elevated serum MMA measurements in detecting patients with overt cobalamin deficiency is reported to be >95%; however, the specificity of this test has not been determined (Hunt et al., 2014). In a study by Rozmarič et al. (2020) the cutoff for MMA as an indicator of B12 deficiency was 0.423 μM with a specificity of 0.90 and sensitivity of 0.91 in newborns; "applying a screening algorithm including only tHCy [total homocysteine] as a second-tier test that may be feasible for many newborn screening labs, newborns with low VitB12, low HoloTC, or elevated MMA can be identified with a positive predictive value between 59% and 87%."

Serum holoTC may be a better indicator of B12-deficiency than serum cobalamin because it represents the biologically active fraction of cobalamin in humans and may be depleted first in subclinical cobalamin deficiency. HoloTC measurements appear to have slighter better sensitivity; however, the specificity of this assay remains to be determined (Oberley & Yang, 2013). It also is not yet clinically validated or available for widespread use (Langan & Goodbred, 2017).

Mak et al. (2023) completed developed a targeted metabolite panel aiming to improve second-tier newborn screening for four inherited metabolic disorders: glutaric acidemia type I, methylmalonic acidemia, ornithine transcarbamylase deficiency, and very long-chain acyl-CoA dehydrogenase deficiency. The panel was assembled from "known disease markers and new features discovered by untargeted metabolomics" and is used to test dried blood samples. The authors completed a validation study on 883 infants. As a second-tier analysis method, the test had 100% screening sensitivity and an 84.5% reduction rate of MMA false positives. The authors conclude that "these findings establish the effectiveness of this second-tier test to improve screening for these four conditions" (Mak et al., 2023).

Criteria	Sensitivity	Specificity	Pitfalls
Serum total cobalamin (<200 pg/mL)	95–97%	Uncertain, possibly <80%	*Elevated levels seen with*:
			Assay technical failure
			Occult malignancy
			Alcoholic liver disease
			Renal disease
			Decreased levels also seen with:
			Haptocorrin deficiency
			Folate deficiency
			Plasma cell myeloma
			HIV
			Pregnancy
Elevated serum methylmalonic acid	>95%	Uncertain	*Elevated levels seen with*:
			Renal insufficiency
			Hypovolemia
			Congenital metabolic defects
			Amyotrophic lateral sclerosis
Elevated serum homocysteine	>95%	Uncertain, less specific than methylmalonic acid	*Elevated levels seen with*:
			Folate or pyridoxine deficiency
			Renal insufficiency
			Hypovolemia
			Hypothyroidism
			Psoriasis
			Congenital metabolic defects
			Neurodegenerative disease
			Malignancy
			Medications
Decreased serum holotranscobalamin	Similar to total cobalamin	Uncertain	*Levels may be affected by*:
			Liver disease
			Macrophage activation
			Autoantibodies

Clinical Utility and Validity

Health Quality Ontario (HQO) performed an extensive meta-analysis of the clinical utility of B12 testing in patients with suspected dementia or cognitive decline because more than 2.9 million serum B12 tests were performed in Ontario alone in 2010 (HQO, 2013). HQO included data from eighteen different studies to address three questions:

1. "Is there an association between vitamin B12 deficiency and the onset of dementia or cognitive decline?
2. Does treatment with vitamin B12 supplementation improve cognitive function in patients with dementia or cognitive decline and vitamin B12 deficiency?
3. What is the effectiveness of oral versus parenteral vitamin B12 supplementation in those with confirmed vitamin B12 deficiency?"

They concluded that "This evidence-based analysis assessed the usefulness of serum vitamin B12 testing as it relates to brain function. This review found very low-quality evidence that suggests a connection between high plasma homocysteine levels (a by-product of B vitamin metabolism in the body) and the onset of dementia. Moderate quality of evidence indicates treatment with vitamin B12 does not improve brain function. Moderate quality of evidence also indicates treatment using oral vitamin B12 supplements is as effective as injections of vitamin B12" (HQO, 2013).

Another meta-analysis, completed in 2015, utilized data from 12 studies and a total of 34,481 patients to determine if vitamin B12, vitamin B6, and folic acid supplementation affected homocysteine levels and/or reduced the risk of cardiovascular disease (Li et al., 2015). A combination of vitamin B12, vitamin B6, and folic acid was found to significantly reduce plasma homocysteine levels, but it did not seem to impact cardiovascular disease risk (Li et al., 2015). Therefore, it was concluded that vitamin B12 should not be utilized as a cardiovascular disease prevention method. Additional research has also concluded that the "Use of vitamin B12 in patients with elevated serum homocysteine levels and cardiovascular disease does not reduce the risk of myocardial infarction or stroke, or alter cognitive decline" (Langan & Goodbred, 2017).

In other indications, vitamin B12 has recently been utilized as a biomarker for patients undergoing therapeutic treatment for tuberculosis (TB); vitamin B12 serum concentrations were observed to have significant differences in TB patients between baseline and six months after anti-TB treatment (ATT), attributing the decrements in vitamin B12 to the body "reclaiming normal physiological function of the affected organs and immune function improv[ing] by cleaning or a rapid drop in bacterial load" (Gebremicael et al., 2019). Gebremicael et al. (2019) also found that HIV (Human Immunodeficiency Virus) and HAART (Highly active antiretroviral therapy) status of TB patients at baseline had "no effect on the concentration levels of vitamin B12 and vitamin A," and HAART treatment did not affect vitamin B12 serum concentration in ATT treated HIV+/TB+ patients.

Wolffenbuttel et al. (2020) recently conducted a study obtaining data from the general population of National Health and Nutrition Examination Survey (NHANES). A total of 24462 patients were included. The authors found a positive association between low serum B12 concentration and all-cause mortality (hazard ratio [HR] = 1.39), as well as between low serum B12 concentration and cardiovascular mortality (HR = 1.64). The authors also found a positive association of high serum B12 concentration and cardiovascular mortality (HR = 1.45), although the authors noted that participants with diagnoses such as hyperlipidemia and CVD tended to use vitamin B12-containing supplements more often than those without such diagnoses. However, the authors did not find an association between vitamin B12 supplement intake and mortality. This demonstrates the importance of testing for B12 in the long run to adjust dietary intake and reduce mortality (Wolffenbuttel et al., 2020).

Sasaki et al. (2023) studied the usefulness of the one-hour ^{13}C-propionate breath test in detecting Vitamin B12. The ^{13}C-propionate breath test can use vitamin B12 as a coenzyme of methylmalonyl-CoA in propionate metabolism to measure vitamin B12 deficiency. The authors collected samples from 49 patients in Japan with clinically suspected vitamin B12 deficiency and compared results between patients with or without low serum vitamin B12 levels, macrocytosis, and vitamin B12 supplementation. The results have no significant difference between the patients with or without low serum VB12 levels. The results did have significant differences between patients with and without macrocytosis and between patients before and after vitamin B12 supplementation (Sasaki et al., 2023).

Guidelines and Recommendations

American Academy of Family Physicians (AAFP)

The AAFP does not recommend screening persons at average risk of vitamin B12 deficiency. Screening should be considered in patients with risk factors, and diagnostic testing should be considered in those with suspected clinical manifestations. These manifestations are listed below:

- "Cutaneous
  - Hyperpigmentation
  - Jaundice
  - Vitiligo
- Gastrointestinal
  - Glossitis
- Hematologic
  - Anemia (macrocytic, megaloblastic)
  - Leukopenia
  - Pancytopenia
  - Thrombocytopenia
  - Thrombocytosis
- Neuropsychiatric
  - Areflexia
  - Cognitive impairment (including dementia-like symptoms and acute psychosis)
  - Gait abnormalities
  - Irritability
  - Loss of proprioception and vibratory sense
  - Olfactory impairment
  - Peripheral neuropathy"

"The recommended laboratory evaluation for patients with suspected vitamin B12 deficiency includes a complete blood count and serum vitamin B12 level." Also, "in patients with a normal or low-normal serum vitamin B12 level, complete blood count results demonstrating macrocytosis, or suspected clinical manifestations, a serum methylmalonic acid level is an appropriate next step and is a more direct measure of vitamin B12's physiologic activity. Although not clinically validated or available for widespread use, measurement of holotranscobalamin, the metabolically active form of vitamin B12, is an emerging method of detecting deficiency."

The AAFP notes that different causes of vitamin B12 deficiency have corresponding "time to improvement" after initiation of treatment. For abnormalities related to "Homocysteine or methylmalonic acid level, or reticulocyte count," AAFP lists an "expected time until improvement" of one

week; for neurologic symptoms, six weeks to three months; for anemia, leukopenia, mean corpuscular volume, or thrombocytopenia, eight weeks.

Finally, AAFP lists risk factors for vitamin B12 deficiency, which are included below:

- "Decreased ileal absorption
  - Crohn disease
  - Ileal resection
  - Tapeworm infection
- Decreased intrinsic factor
  - Atrophic gastritis
  - Pernicious anemia
  - Postgastrectomy syndrome (includes Roux-en-Y gastric bypass)
- Genetic
  - Transcobalamin II deficiency
- Inadequate intake
  - Alcohol abuse
  - Patients older than 75 years
  - Vegans or strict vegetarians (including exclusively breastfed infants of vegetarian/vegan mothers)
- Prolonged medication use
  - Histamine H2 blocker use for more than 12 months.
  - Metformin use for more than four months
  - Proton pump inhibitor use for more than 12 months" (Langan & Goodbred, 2017).

The AAFP comments on pernicious anemia, stating that "Patients diagnosed with vitamin B_{12} deficiency whose history and physical examination do not suggest an obvious dietary or malabsorptive etiology should be tested for pernicious anemia with anti-intrinsic factor antibodies (positive predictive value = 95%), particularly if other autoimmune disorders are present." The AAFP also notes that "Patients with pernicious anemia may have hematologic findings consistent with normocytic anemia" (Langan & Goodbred, 2017).

In their "Update on Vitamin B12 Deficiency" published in the *American Family Physician*, Langan and Zawistoski (2011) remarked that "No major medical organizations, including the U.S. Preventive Services Task Force, have published guidelines on screening asymptomatic or low-risk adults for vitamin B12 deficiency, but high-risk patients, such as those with malabsorptive disorders, may warrant screening" (Langan & Zawistoski, 2011).

American College of Gastroenterology (ACG)

The ACG Clinical Guidelines: Diagnosis and Management of Celiac Disease (Rubio-Tapia et al., 2013) state that "tissue transglutaminase and deamidated gliadin peptide can be used for monitoring CD [celiac disease]. Other tests may include complete blood count, alanine aminotransferase, vitamins (A, D, E, B12), copper, zinc, carotene, folic acid, ferritin, and iron. Blood tests at follow-up should be individualized to verify correction of laboratory tests that were abnormal at baseline" (Rubio-Tapia et al., 2013).

Centers for Disease Control and Prevention (CDC)

The CDC emphasizes the importance of vitamin B12 for infants' healthy development. Infants may acquire sufficient vitamin B12 through breastmilk; however, if a breastfeeding mother is deficient in vitamin B12, the mother's infant may not receive enough of the vitamin. The CDC states breastfeeding mothers who have had a malabsorptive bariatric procedure (such as gastric bypass surgery), who have pernicious anemia (low number of red blood cells caused by a deficiency of vitamin B12), or who have certain gastrointestinal disorders, may not be able to absorb various vitamins and minerals, such as vitamin B12, folic acid (vitamin B9), iron, and calcium. "Healthcare providers should monitor these mothers for nutrient deficiencies, including vitamin B12 deficiency" (CDC, 2024).

British Committee for Standards in Haematology

The British Committee for Standards in Haematology guidelines for the diagnosis and treatment of cobalamin and folate disorders state that: "Serum cobalamin currently remains the first-line test, with additional second-line plasma methylmalonic acid to help clarify uncertainties of underlying biochemical/functional deficiencies. Serum holotranscobalamin has the potential as a first-line test, but an indeterminate 'grey area' may still exist. Plasma homocysteine may be helpful as a second-line test but is less specific than methylmalonic acid. The availability of these second-line tests is currently limited" (Devalia et al., 2014).

The Doctors of BC (formerly the British Columbia Medical Association)

The Doctors of BC updated their guidelines on vitamin B12 in 2023. The guidelines key recommendations are:

- "Routine B12 screening and testing in asymptomatic patients is not supported by evidence.
- Consider B12 supplementation without testing in asymptomatic patients with risk factors for B12 deficiency.
- B12 deficiency can cause preventable permanent injury and should be considered with new onset neurological conditions and symptoms suggestive of B12 deficiency.
- Folate testing is rarely indicated but may be available via consultation with the laboratory medicine physician or scientist" (BCMA, 2023).

The guidelines go on to state: "In a clinically symptomatic patient with specific features of B12 deficiency, order a B12 test." In terms of repeat testing, the guidelines state that "Repeat testing of B12 may be warranted after a trial of therapy or as an assessment of adherence. Repeat testing should wait at least 2 months after therapy has been started. If the B12 is normal (rare probability of B12 deficiency – see Table 3: B12 Medication Table), a repeat investigation is not required in the absence of new signs of disease. In absence of a reversible factor therapy, supplementation in most cases is lifelong." Lastly, the guidelines state that "Serum folate and red blood cell (RBC) folate testing is no longer offered in BC." (BCMA, 2023).

American Association of Clinical Endocrinology (AACE), the American College of Endocrinology (ACE) and The Obesity Society (TOS)

"Vitamin B12 levels should be checked periodically in older adults and patients on metformin therapy (Grade A, BEL 1). With the exception of early treatment of patients with neurologic symptoms, pernicious anemia, or malabsorptive bariatric surgery requiring parenteral (intramuscular or subcutaneous) vitamin B12 replacement, patients with vitamin B12 deficiency can generally be treated with oral vitamin B12 (1,000 µg per day of oral crystalline cobalamin) and may benefit from increasing the intake of vitamin B12 in food (Grade A, BEL 1)" (Gonzalez-Campoy et al., 2013).

American Association of Clinical Endocrinology (AACE) and the American College of Endocrinology (ACE)

In a consensus statement on the Comprehensive Type 2 Diabetes Management Algorithm, the AACE/ACE states that "in patients taking metformin who develop neuropathy, B12 should be monitored and supplements given to affected patients, if needed" (Garber et al., 2020).

American Society for Metabolic and Bariatric Surgery (ASMBS) Integrated Health Nutritional Guidelines (2016 Update)

Concerning vitamin B12 screening and weight loss surgical (WLS) practices, the ASMBS states that "routine pre-WLS screening of B12 is recommended for all patients (Grade B, BEL 2)." Further, serum MMA [methylmalonic acid] testing is recommended to evaluate a possible B12 deficiency for both asymptomatic and symptomatic patients as well as in "those with history of B12 deficiency or preexisting neuropathy (Grade B, BEL 2)"

The ASMBS also makes the following recommendations for post-WLS nutrient screening:

- "Routine post-WLS screening of vitamin B12 status is recommended for patients who have undergone RYGB [Roux-en-Y gastric bypass], SG [sleeve gastrectomy], or BPD/DS [biliopancreatic diversion/duodenal switch]."
- "More frequent screening (e.g., every 3 mo) is recommended in the first post-WLS year, and then at least annually or as clinically indicated for patients who chronically use medications that exacerbate risk of B12 deficiency: nitrous oxide, neomycin, metformin, colchicine, proton pump inhibitors, and seizure medications."
- "Serum B12 may not be adequate to identify B12 deficiency. It is recommended to include serum MMA with or without homocysteine to identify metabolic deficiency of B12 in symptomatic and asymptomatic patients and in patients with history of B12 deficiency or preexisting neuropathy." (Parrott et al., 2017).

American Association of Clinical Endocrinology/American College of Endocrinology (AACE/ACE), The Obesity Society (TOS), American Society for Metabolic & Bariatric Surgery (ASMBS), Obesity Medicine Association (OMA), and American Society of Anesthesiologists (ASA) (2019 Update)

The AACE/ACE, TOS, ASMBS, OMA, and ASA published clinical practice guidelines for perioperative nutrition, metabolic, and nonsurgical support of patients undergoing bariatric procedures in 2019. In the preprocedural checklist, the recommendation includes "nutrient screening with iron studies, B12 and folic acid (RBC folate, homocysteine, methylmalonic acid optional), and 25-vitamin D (vitamins A and E optional); consider more extensive testing in patients undergoing malabsorptive procedures based on symptoms and risks." In the post-procedure checklist, for early postoperative care, vitamin B12 should be assessed "as needed for normal range levels," and in follow-up "annually; MMA and HCy optional; then q 3-6 months if supplemented)" (Mechanick et al., 2019). In addition, the societies state:

- Vitamin B12 screening is "recommended for patients who have undergone RYGB [Roux-en-Y gastric bypass], SG [sleeve gastrectomy], or BPD/DS (biliopancreatic diversion/duodenal switch]"
- "Patients who become pregnant following bariatric procedure should have nutritional surveillance and laboratory screening for nutrient deficiencies every trimester, including iron, folate, vitamin B12, vitamin D, and calcium, and if after a malabsorptive procedure, fat-soluble vitamins, zinc, and copper (Grade D)

- Baseline and annual post-bariatric procedure evaluation for vitamin B12 deficiency should be performed in all patients (Grade B; BEL 2)
- More frequent aggressive case finding (e.g., every 3 months) should be performed in the first postoperative year, and then at least annually or as clinically indicated for patients who chronically use medications that exacerbate risk of B12 deficiency: nitrous oxide, neomycin, metformin, colchicine, proton-pump inhibitors, and seizure medications (Grade B, BEL 2)
- Since serum B12 may not be adequate to identify B12 deficiency, consider measuring serum methylmalonic, with or without homocysteine, to identify a metabolic deficiency of B12 in symptomatic and asymptomatic patients and in patients with a history of B12 deficiency or pre-existing neuropathy (Grade B, BEL 2)
- B12 status should be assessed in patients on higher-dose folic acid supplementation (>1000 µg/day) to detect a masked B12- deficiency state (Grade D)" (Mechanick et al., 2019).

American Society for Metabolic & Bariatric Surgery (ASMBS)

Pratt et al. (2018) state that "Anemia is common after MBS [metabolic and bariatric surgery] and may relate to low levels of iron, folate, B6, or B12. Dieticians with expertise in MBS are best equipped to assess nutritional status, including screening for frank nutrient deficiencies." Further, "preparation for MBS educates patients and families to the importance of taking vitamins and supplements regularly before MBS to reduce the risk of deficiencies after MBS. Preoperative nutritional assessment includes serum iron, folate, ferritin, and total iron-binding capacity (TIBC); thiamin (B1); vitamin B12; vitamin A and B6; calcium, Parathyroid Hormone, alkaline phosphatase, vitamin D, phosphorus, magnesium, and zinc. All except serum magnesium and zinc should be checked 2 months post surgery and all should be checked at 6 months and then yearly thereafter." Finally, "standard supplementation recommended for adolescents includes vitamin B1 preoperatively and for at least 6 months postoperatively, vitamin B12 sublingual, multivitamin with iron, and calcium citrate with vitamin D daily" (Pratt et al., 2018).

British Obesity & Metabolic Surgery Society (BOMSS) (2020 Update)

The BOMSS released 2020 perioperative and postoperative guidelines on biochemical monitoring and micronutrient replacement for patients undergoing bariatric surgery. On measuring vitamin B12 concentrations, the BOMSS has included checking a "full blood count including haemoglobin, ferritin, folate and vitamin B12 levels" in their preoperative nutritional assessment with a grade B and evidence level (EL) of 2. For postoperative care and biochemical monitoring, the BOMSS stated,

- "Check vitamin B12 levels at regular intervals following SG, RYGB and malabsorptive procedures such as BPD/DS" (Grade B, EL2).
- Consider the following frequency of monitoring vitamin B12 levels: 3, 6 and 12 months in the first year and at least annually thereafter so that changes in status may be detected" (GPP – good practice point)."

With relation to folic acid deficiency, O'Kane et al. (2020) mentions, "check and treat for vitamin B12 deficiency, before initiating folic acid treatment to avoid precipitation of subacute combined degeneration of the spinal cord" (Grade D, EL4). For any presence of neurological symptoms/Wernicke's encephalopathy, the guidelines recommend to "check for vitamin B12, copper and vitamin E deficiencies and treat" (GPP). In pregnant individuals after undergoing bariatric surgery, checking for vitamin B12 deficiency, among other nutritional deficiencies, has been recommended for each trimester and prior to additional folic acid supplementation in the preconception period (O'Kane et al., 2020).

Guidelines for Diagnosis and Management of the Cobalamin-related Remethylation Disorders cblC, cblD, cblE, cblF, cblG, cblJ and MTHFR Deficiency

This international consortium of scientists from Europe and the U.S. issued guidelines "within the frame of the 'European network and registry for homocystinurias and methylation defects' (E-HOD) project." For Recommendation 5, they state (Quality of the evidence: moderate), "we strongly recommend that in the case of high total homocysteine, plasma and urine samples for determination of MMA, methionine, folate and vitamin B12 are to be obtained before treatment is started" (Huemer et al., 2017).

The American Diabetes Association (ADA)

The ADA states that in patients with type 2 diabetes, the long-term use of metformin may be associated with a vitamin B12 deficiency; therefore, a Grade B recommendation has been made that recommends considering "periodic assessment of vitamin B12 level in those taking metformin chronically should be considered to check for possible deficiency"(American Diabetes Association Professional Practice, 2023a). In 2024, the ADA stated that "Measurement of vitamin B12 levels should be considered for patients with type 1 diabetes and peripheral neuropathy or unexplained anemia" (American Diabetes Association Professional Practice, 2023b).

American Psychiatric Association (APA)

The APA released guidelines that state that vitamin B12 deficiencies can develop due to anorexia nervosa, atypical anorexia nervosa, or avoidant restrictive food intake disorder (APA, 2023).

The Vitamin B12 Consensus Panelists Group

A consensus group of experts developed a series of recommendations on Vitamin B12 screening and diagnosis (Obeid et al., 2024). The following recommendations pertain to biomarkers and their utility in clinical practice:

"Considering the cost–benefit and the added value of advanced laboratory tests beyond plasma B12 concentrations and blood cell count:

- Measurement of a metabolic marker such as plasma methylmalonic acid (or total homocysteine if methylmalonic acid is not available) is useful in guiding the diagnosis of B12 deficiency.
- If available, plasma methylmalonic acid concentration is a useful marker for monitoring the effectiveness of B12 treatment in general.
- If available, plasma methylmalonic acid concentration is useful in monitoring the success of oral B12 treatment in particular when it is questionable whether the B12 dose is appropriate or people can absorb B12.
- Plasma methylmalonic acid concentration (or at least total homocysteine) should be made available for all people suspected of having B12 deficiency.
- Although the metabolic markers (plasma methylmalonic acid and total homocysteine) have some limitations, they can be very helpful when the clinical picture is uncertain.
- Because chronic use of metformin in patients with diabetes is associated with lower plasma concentrations of B12 and linked to the frequency and severity of neuropathy, measurement of B12 status once per year in this group of patients can help detecting a deficiency prior to clinical manifestation.
- If plasma B12 concentrations far above the reference range are encountered in a person without specific medical conditions:

    - Inquire if the person is using any supplemental B12 source (food supplements or OTC).
    - If the person is not using a B12-supplement, repeat plasma B12 test after few months.
    - Rule out disturbed blood count, liver and renal function markers that may explain high plasma B12 levels due to liver or kidney diseases or undiagnosed malignancies
- In context of the B12 diagnostic work-up, folate and iron status should also be assessed" (Obeid et al., 2024).

National Institute for Health and Care Excellence (NICE)

The National Institute for Health and Care Excellence (NICE) provides guidelines on the diagnosis and management of vitamin B12 deficiency, emphasizing targeted testing based on specific symptoms and risk factors to improve diagnostic accuracy. Testing is advised when patients display both a common symptom and a common risk factor for B12 deficiency. Clinical judgment is advised in cases where a patient presents only symptoms and no risk factors (NICE, 2024). Common symptoms and common risk factors are outlined below:

"Common symptoms and signs of vitamin B12 deficiency

- abnormal findings on a blood count such as anaemia or macrocytosis
- cognitive difficulties such as difficulty concentrating or short-term memory loss (sometimes described as 'brain fog'), which can also be symptoms of delirium or dementia
- eyesight problems related to optic nerve dysfunction:
    - blurred vision
    - optic atrophy
    - visual field loss (scotoma)
- glossitis
- neurological or mobility problems related to peripheral neuropathy, or to central nervous system disease including myelopathy (spinal cord disease):
    - balance issues and falls caused by impaired proprioception (the ability to sense movement, action and location) and linked to sensory ataxia (which may have been caused by spinal cord damage)
    - impaired gait
    - pins and needles or numbness (paraesthesia)
- symptoms or signs of anaemia that suggest iron treatment is not working properly during pregnancy or breastfeeding
- unexplained fatigue.

Common risk factors for vitamin B12 deficiency

- diet low in vitamin B12 (without the regular use of over-the-counter preparations), for example, in people who:
    - follow a diet that excludes, or is low in, animal-source foods (such as a vegan diet, or diets excluding meat for religious beliefs)
    - do not consume food or drinks fortified with vitamin B12
    - have an allergy to some foods such as eggs, milk or fish
    - find it difficult to buy or prepare food (for example, people who have dementia or frailty, or those with mental health conditions)
    - find it difficult to obtain or afford foods rich in vitamin B12 (for example, people on low income)
    - have a restricted diet (for example, because of an eating disorder)
- family history of vitamin B12 deficiency or an autoimmune condition

- health conditions:
  - atrophic gastritis affecting the gastric body
  - coeliac disease or another autoimmune condition (such as thyroid disease, Sjögren's syndrome or type 1 diabetes)
- medicines:
  - colchicine
  - H2-receptor antagonists
  - metformin (see the MHRA safety advice on metformin and reduced vitamin B12)
  - phenobarbital
  - pregabalin
  - primidone" (NICE, 2024)

Regarding initial testing, NICE recommends "either total B12 (serum cobalamin) or active B12 (serum holotranscobalamin) as the initial test for suspected vitamin B12 deficiency unless the test needs to be done during pregnancy, or recreational nitrous oxide use is the suspected cause of deficiency"(NICE, 2024).

For individuals with indeterminate test results who show no symptoms, NICE advises them to seek care if symptoms develop. If the initial test result suggests that a deficiency is unlikely, NICE recommends that "if they are still experiencing symptoms 3 to 6 months later, consider a repeat of the initial test" (NICE, 2024).

The British Medical Journal (BMJ)

The British Medical Journal (BMJ) published a 2023 study on diagnosing and managing vitamin B12 deficiency. Regarding testing, the BMJ notes, "Diagnosing B12 deficiency can be challenging because no single specific measurement exists to reliably diagnose or refute the presence of B12 deficiency." For patients with neurological symptoms, the BMJ states, "Serum B12, homocysteine, and methylmalonic acid (MMA) levels are unreliable predictors of B12 responsive neuropathy" (Wolffenbuttel et al., 2023).

The BMJ also highlights, "Neurological symptoms may take several months or even years to resolve completely," and that "Biomarkers normalize more rapidly than an improvement or reversal of (neurological) symptoms. Additionally, symptoms may reappear without changes in biomarker status." For treatment, they recommend that providers "monitor symptoms regularly (eg, every two to three months) as they may reappear, even after several months, if injection frequency is reduced. Measuring serum biomarkers such as B12 or MMA is neither helpful nor indicated in assessing or monitoring clinical improvement. Base the injection frequency on symptoms, and not on biomarker assessment" (Wolffenbuttel et al., 2023).

In 2024, the BMJ reviewed the NICE guideline for vitamin B12 deficiency diagnosis and management, agreeing with the recommendations for testing. They noted that "Vitamin B12 deficiency is a complex condition with significant variability in the type and severity of symptoms that people experience". This led to discussion on which features and risk factors should be emphasized, with the guideline committee prioritizing "the importance of timely testing while not increasing the volume of testing unnecessarily in people who are unlikely to be deficient"(Sands et al., 2024).

Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations

(NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

The FDA has cleared numerous devices including needles, reagents, instrumentation, and imaging systems for use in prostate biopsy. Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
82607	Cyanocobalamin (Vitamin B-12)
83090	Homocysteine
83921	Organic acid, single, quantitative
84999	Unlisted chemistry procedure

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

Evidence-based Scientific References

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Revision History

Revision Date	Summary of Changes
12/04/2024	Reviewed and Updated: Updated background, guidelines, and evidence-based scientific references. Literature review necessitated the following changes in coverage criteria: Added a three month testing frequency for all vitamin B testing/screening. Results in a new main criterion 1 and former CC1, CC2, and CC3 before subcriteria under the main criterion, which reads: "1) Total vitamin B12 (serum cobalamin) testing MEETS COVERAGE CRITERIA once every three months for any of the following situations:" New CC7: "7) For all other situations not described above, total vitamin B12 (serum cobalamin) testing DOES NOT MEET COVERAGE CRITERIA.

Vitamin D Testing

Policy Number: AHS – G2005 – Vitamin D Testing	Initial Presentation Date: 11/16/2015 Effective Date: 4/1/2025

Policy Description

Vitamin D is a precursor to steroid hormones and plays a key role in calcium absorption and mineral metabolism. Vitamin D promotes enterocyte differentiation and the intestinal absorption of calcium. Other effects include a lesser stimulation of intestinal phosphate absorption, suppression of parathyroid hormone (PTH) release, regulation of osteoblast function, osteoclast activation, and bone resorption (Pazirandeh & Burns, 2023).

Vitamin D is present in nature in two major forms. Ergocalciferol, or vitamin D2, is found in fatty fish (e.g., salmon and tuna) and egg yolks, although very few foods naturally contain significant amounts of vitamin D. Cholecalciferol, or vitamin D3, is synthesized in the skin via exposure to ultraviolet radiation present in sunlight. Some foods are also fortified with vitamin D, most notably milk and cereals (Sahota, 2014).

Though the risk of vitamin D deficiency can differ by age, sex, and race and ethnicity, major risk factors for vitamin D deficiency include inadequate sunlight exposure, inadequate dietary intake of vitamin D-containing foods, and malabsorption syndromes, such as Crohn's disease and celiac disease (Dedeoglu et al., 2014; Looker et al., 2011).

Related Policies

Policy Number	Policy Title

AHS-G2164	Parathyroid Hormone, Phosphorus, Calcium, and Magnesium Testing

Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) For individuals with an underlying disease or condition which is specifically associated with vitamin D deficiency or decreased bone density (see Note 1) or for individuals suspected of hypervitaminosis of Vitamin D, 25-hydroxyvitamin D serum testing **MEETS COVERAGE CRITERIA**.

2) As part of the total 25-hydroxyvitamin D analysis, testing for D2 and D3 fractions of 25-hydroxyvitamin D **MEETS COVERAGE CRITERIA**.

3) For individuals who have documented vitamin D deficiency, repeat testing for serum 25-hydroxyvitamin D at least 12 weeks after the initiation of vitamin D supplementation therapy **MEETS COVERAGE CRITERIA** with the following restrictions:

a) Twice per year testing for the monitoring of supplementation therapy, until the therapeutic goal has been achieved.

b) Annual testing once the therapeutic range has been achieved.

4) For the evaluation or treatment of conditions that are associated with defects in vitamin D metabolism (see Note 2), 1,25-dihydroxyvitamin D serum testing **MEETS COVERAGE CRITERIA**.

5) The following testing **DOES NOT MEET COVERAGE CRITERIA**:

a) Measurement of serum 1,25-dihydroxyvitamin D to screen for vitamin D deficiency.

b) Routine screening for vitamin D deficiency with serum testing in asymptomatic individuals and/or during general encounters.

NOTES:

Note 1: Indications for serum measurement of 25-hydroxyvitamin D are as follows:

A. Biliary cirrhosis and other specified disorders of the biliary tract
B. Blind loop syndrome
C. Celiac Disease
D. Coronary artery disease in individuals where risk of disease progression is being considered against benefits of chronic vitamin D and calcium therapy
E. Dermatomyositis
F. Eating disorders
G. Having undergone, or for those who have been scheduled for, bariatric procedures such as Roux-en-Y gastric bypass, sleeve gastrectomy, or biliopancreatic diversion with or without duodenal switch
H. Hypercalcemia, hypocalcemia, or other disorders of calcium metabolism
I. Hyperparathyroidism or hypoparathyroidism

J. Individuals receiving hyperalimentation
K. Inflammatory bowel disease (Crohn's disease and ulcerative colitis)
L. Intestinal malabsorption
M. Liver cirrhosis
N. Long term use of anticonvulsants, glucocorticoids and other medications known to lower vitamin D levels
O. Malnutrition
P. Myalgia and other myositis not specified
Q. Myopathy related to endocrine diseases
R. Neoplastic hematologic disorders
S. Osteogenesis imperfecta
T. Osteomalacia
U. Osteopetrosis
V. Osteoporosis
W. Pancreatic steatorrhea
X. Primary or miliary tuberculosis
Y. Psoriasis
Z. Regional enteritis
AA. Renal, ureteral, or urinary calculus
BB. Rickets
CC. Sarcoidosis
DD. Stage III-V Chronic Kidney Disease and End Stage Renal Disease
EE. Systemic lupus erythematosus

Note 2: Indications for serum testing of 1,25-dihydroxyvitamin D are as follows:

A. Disorders of calcium metabolism
B. Familial hypophosphatemia
C. Fanconi syndrome
D. Hyperparathyroidism or hypoparathyroidism
E. Individuals receiving hyperalimentation
F. Neonatal hypocalcemia
G. Osteogenesis imperfecta
H. Osteomalacia
I. Osteopetrosis
J. Primary or miliary tuberculosis
K. Renal, ureteral, or urinary calculus
L. Rickets
M. Sarcoidosis
N. Stage III-V Chronic Kidney Disease and End Stage Renal Disease

Table of Terminology

Term	Definition
25(OH)D	25-Hydroxyvitamin D
25OHD	25-Hydroxyvitamin D
AACE	American Association of Clinical Endocrinologists
ACE	American College of Endocrinology
AAP	American Academy of Pediatrics

ACOG	American College of Obstetricians and Gynecologists
BMD	Bone mineral density
BPD	Biliopancreatic diversion
CDC	The Centers for Disease Control and Prevention
CLIA '88	Clinical Laboratory Improvement Amendments of 1988
CMS	Centers for Medicare & Medicaid Services
CV	Coefficient of variation
DS	Duodenal switch
ELISA	Enzyme-linked immunosorbent assay
ES	Endocrine Society
FDA	Food and Drug Administration
HPLC	High-performance liquid chromatography
IDS	Immunodiagnostic Systems
IOM	Institute of Medicine
IU	International unit
LAGB	Laparoscopic adjustable gastric banding
LC/MS	Liquid chromatography coupled with mass spectrometry
LDTs	Laboratory developed tests
LSG	Lennox-Gastaut Syndrome
MEWS	Modified Early Warning Score
MS	Mass spectrometry
NHANES	National Health and Nutrition Examination Surveys
PCR	Polymerase chain reaction
PTH	Parathyroid hormone
RIA	Radioimmunoassay
ROS	Royal Osteoporosis Society
RYGB	Rouxen-Y gastric bypass
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SPF	Sun protection factor
THIN	The Health Improvement Network
USPSTF	United States Preventive Services Task Force
UVB	Ultraviolet B
VDSCP	Vitamin D Standardization Certification Program

Scientific Background

Vitamin D is an important nutrient that helps the body absorb calcium and maintain adequate bone strength. In order to be used in the metabolic process, vitamin D that is consumed or formed in the skin must first be activated via the addition of hydroxyl groups. Two forms of activated vitamin D are found in human circulation: 25-hydroxyvitamin D (calcidiol or 25OHD) and 1,25-dihydroxyvitamin D (calcitriol). 25-hydroxyvitamin D is the predominant and most stable form, but 1,25-dihydroxyvitamin D is the metabolically active form. The initial activation step occurs in the liver, where 25OHD is synthesized, and the second hydroxyl group is added in the kidney, creating the fully activated 1,25-dihdroxy form (Sahota, 2014).

25-hydroxyvitamin D has a half-life of 15 days in the circulation, whereas 1,25-dihydroxyvitamin D has a much shorter circulating half-life of 15 hours. Consequently, measurement of serum 25OHD is generally accepted as the preferred test to evaluate an individual's vitamin D status despite lack of standardization between methods and laboratories (Glendenning & Inderjeeth, 2012; Sahota, 2014; Scott et al., 2015).

Vitamin D deficiency typically is defined as a serum 25OHD level less than 20 ng/ml, and certain organizations consider <30 ng/ml as insufficient. Trials of vitamin D supplementation (Chapuy et al., 2002; Dawson-Hughes et al., 1997; Sanders et al., 2010; Trivedi et al., 2003) and the Institute of Medicine (IOM) systematic review (Ross et al., 2011) recommend maintaining the serum 25OHD concentration between 20 and 40 ng/mL (50 to 100 nmol/L), whereas other experts favor maintaining 25OHD levels between 30 and 50 ng/mL (75 to 125 nmol/L). Experts agree that levels lower than 20 ng/mL are suboptimal for skeletal health. The optimal serum 25OHD concentrations for extra-skeletal health have not been established (Dawson-Hughes, 2024). Approximately 15% of the U.S. pediatric population suffers from either vitamin D deficiency or insufficiency. Limited sun exposure and the use of sunscreen compromises production of vitamin D, contributing to low 25OHD levels. "UVB absorption is blocked by artificial sunscreens, and sunscreens with a sun protection factor (SPF) of 30 can decrease vitamin D synthetic capacity by as much as 95 percent" (Madhusmita, 2024). Also, "vitamin D deficiency has been reported in dark-skinned immigrants from warm climates to cold climates in North America and Europe" (Dawson-Hughes, 2023). For example, a study by Awumey and colleagues found that Asian Indians who immigrated to the U.S. were considered vitamin D insufficient or deficient even after the administration of 25OHD. "Thus, Asian Indians residing in the U.S. are at risk for developing vitamin D deficiency, rickets, and osteomalacia" (Awumey et al., 1998).

Vitamin D deficiency has been associated with important short and long term health effects, such as rickets, osteomalacia, and the risk of osteoporosis (Sahota, 2014). Rickets in children can result in skeletal deformities. To prevent nutritional rickets in infants, vitamin D supplementation is recommended at 400 IU/day; personalized dosages are possible and would require 25OHD testing (Zittermann et al., 2019). In adults, osteomalacia can result in muscular weakness, bone weakness, and osteoporosis which leads to an increased risk for falls and fractures (Granado-Lorencio et al., 2016).

A role for vitamin D has been suggested in several other conditions and metabolic processes including, but not limited to, cancer, cardiovascular disease, hypertension, diabetes, and preeclampsia. While vitamin D insufficiency has been associated with several cancer types, inconsistencies cause discrepancies in suggested treatment methods; currently, no official institutional guidelines recommend a dietary vitamin D supplementation for cancer prevention (McNamara & Rosenberger, 2019). 25-hydroxyvitamin D (25OHD) is the accepted biomarker of circulating vitamin D, and in utilization of this biomarker, researchers have reported an association between a high vitamin D production rate and a lowered risk of colorectal cancer (Weinstein et al., 2015). Further, low concentrations of 25OHD have been associated with a high risk of cardiovascular disease and mortality, suggesting that patients deficient in vitamin D have an increased risk in developing cardiovascular disease (Crowe et al., 2019). However, conclusive evidence for the role of vitamin D in these conditions is not available (Aspray et al., 2014; Ross et al., 2011). Based on controversial evidence, researchers continue to emphasize the fact that vitamin D supplementation is not an accepted prevention method for cardiac events or cancer (Ebell, 2019).

Certain other conditions may impact an individual's ability to absorb or activate vitamin D, thereby resulting in vitamin D deficiency. These include, but are not limited to, Crohn's disease, ulcerative colitis, celiac disease, liver cirrhosis, chronic kidney disease, and bariatric surgery. Since Vitamin D is fat soluble, any impact on fat absorption or storage may affect circulating vitamin D levels (Dawson-Hughes, 2023; Fletcher et al., 2019).

According to the Institute of Medicine (IOM), routine dietary supplementation with vitamin D is recommended for most individuals. While there are no differences regarding gender and recommended daily dose of vitamin D, there are differences depending on age. The IOM recommends a dietary allowance of 600 IU for individuals up to 70 years old, and 800 IU for individuals older than 70 (Ross et al., 2011), although these recommendations have been met with some criticism as being too low to adequately impact vitamin D levels in some individuals. The USPSTF recommends against daily supplementation with 400 IU or less of vitamin D3 and 1000 mg or less of calcium for the primary prevention of fractures in noninstitutionalized postmenopausal individuals (Moyer, 2013).

Vitamin D toxicity is very rare and occurs only when levels of 25OHD are >500 nmol/L [>200 ng/mL], which is well above the level considered sufficient. Vitamin D toxicity may cause hypercalciuria, hypercalcemia, renal stones, and renal calcification with renal failure (Moyer, 2013). Additional research suggests that excess 25-hydroxyvitamin D3 aggravates tubulointerstitial injury (Kusunoki et al., 2015).

Insource Diagnostics has developed two similar quantitative laboratory developed tests (LDTs) termed Sensieva Vena™ 25OH Vitamin D2/D3 and Droplet 25OH Vitamin D2/D3. These assays utilize liquid chromatography coupled with mass spectrometry (LC/MS/MS) to measure both D2 and D3. The LC/MS/MS assessment technique is the apparent gold standard for vitamin D2 and D3 measurement, and is the only currently available method to measure both vitamins individually. These assays may assist in the measurement of several ailments related to abnormal vitamin D levels including parathyroid function, dietary absorption, calcium metabolism, and vitamin D treatment effectiveness; serum, plasma and blood microsamples can be utilized for these tests. The 20uL serum/plasma method of the Sensieva™ 25OH Vitamin D2/D3 LDT was approved by the CDC's VDSCP in 2017-2018 (CDC, 2019). This test is no longer certified by the CDC's VDSCP and as of May 2020 Insource Diagnostics website has been removed. Therefore, it is unclear if this test is still available.

Analytical Validity

Serum or plasma concentration of 25OHD can be measured using several assays, including ELISA, radioimmunoassay (RIA), mass spectrometry, and HPLC. Assays using LC-MS/MS can differentiate between D2 and D3. These methods "can individually quantitate and report both analytes, in addition to providing a total 25-hydroxyvitamin D concentration" (Krasowski, 2011). RIA-based assays for 25OHD can have intra- and inter-assay variations of 8 – 15%, and the Immunodiagnostic Systems (IDS)-developed RIA has a reported 100% specificity for D3 and 75% for D2 (Holick, 2009). "For most HPLC and LC-MS/MS methods extraction and procedural losses are corrected for by the inclusion of an internal standard which, in part, may account for higher results compared to immunoassay" (Wallace et al., 2010). Even though LC-MS/MS is considered to be the gold standard of measuring 25OHD and its metabolites, only approximately 20% of labs report using it (Avenell et al., 2018). One study reports that 46% of samples measured using LC-MS/MS were classified as vitamin D-deficient whereas, when the samples were measured using an immunoassay method, 69% were vitamin D-deficient (<30 nmol/L) (Annema et al., 2018).

The Centers for Disease Control and Prevention (CDC) have developed a vitamin D standardization certification program (VDSCP). This program helps to ensure that all LDT vitamin D tests are accurate and reliable by evaluating the performance and overall reliability of these assessments over time, supplying reference measurements for both 25-hydroxyvitamin D2 and 25-hydroxyvitamin D3, and providing technical support to additional programs and studies (CDC, 2017).

Due to the great variability among the different assays used to measure vitamin D levels, the VDSCP was created. Interassay variability yields an inadequate basis to establish if 25OHD increases or decreases the risk of non-skeletal diseases and hampers the development of evidence-based guidelines and policies (Sempos & Binkley, 2020). VDSCP studies can either be retrospective or prospective; therefore, standardization of national nutrition survey data may be performed. For example, it was originally thought, based on reports from the National Health and Nutrition Examination Surveys (NHANES), that there had been a dramatic decline in mean 25OHD levels in the US population from 1990 to the period 2001–2004. DiaSorin Radioimmunoassay was used to measure 25OHD levels in these surveys. However, after standardizing the results using VDSCP methods, it was found that the mean 25OHD levels were stable from 1990-2004 (Sempos et al., 2018). The VDSCP program established four steps to achieve standardization, as described by:

1. "Fit for use…means that assay chosen will perform appropriately and provide standardised measurements in the patient/study populations in the conditions for which it will be used…[as] some immunoassays do not function appropriately in all patient populations.
2. [Assay is] Certified by the CDC Vitamin D Standardization Certification Program as being standardised and having an appropriate measurement range or be a documented standardised laboratory-developed HPLC or LC-MS/MS assay with an appropriate measurement range…see which ones are currently, or have been in the past, certified by the CDC as meeting VDSP performance criteria of having a total (coefficient of variation) CV≤10% and a mean bias with the range of −5 to +5%... VDSP recommends using an assay that does have an appropriate measurement range for the population it will be used in; for example, it should be able to measure 25(OH)D in persons who are deficient.
3. Appropriate level of assay precision and accuracy…it has been recommended that a standardised LC-MS/MS assay be selected.
4. [The assay] Meets VDSP assay standardisation criteria in your 'hands' or laboratory…. We recommend a testing period in order to verify that an immunoassay is standardized especially since there is generally very little an individual laboratory can do to 'calibrate' an immunoassay" (Sempos & Binkley, 2020).

Clinical Utility and Validity

A retrospective study of 32,363 tests of serum 25OHD found that a significant proportion of the lab requests were unjustified by medical criteria, and "that clinical and biochemical criteria may be necessary to justify vitamin D testing but not sufficient to indicate the presence of vitamin D deficiency" (Granado-Lorencio et al., 2016).

The table below lists the criteria used for vitamin D testing in the study by Granado and colleagues (Granado-Lorencio et al., 2016).

A meta-analysis study by Bolland et al. (2018) of 81 randomized controlled trials with a combined total of 53,537 participants measured the effects, if any, vitamin D supplementation had on fractures, falls, and bone density. They found that there was no clinically relevant difference in bone mineral density at any site between the control and experimental groups; moreover, "for total fracture and falls, the effect estimate lay within the futility boundary for relative risks of 15%, 10%, 7.5%, and 5% (total fracture only), suggesting that vitamin D supplementation does not reduce fractures or falls by these amounts. Our findings suggest that vitamin D supplementation does not prevent fractures or falls or clinically meaningful effects on bone mineral density. There were no differences between the effects of higher and lower doses of vitamin D. There is little justification to use vitamin D supplements to maintain or improve musculoskeletal health. This conclusion should be reflected in clinical guidelines" (Bolland et al., 2018).

A prospective study by Hao et al. (2020) aims to determine whether 25OHD levels is associated with mortality or the ability to walk in a patient cohort after hip fracture surgery. Each year, 319,000 elderly patients, are hospitalized for hip fractures (CDC, 2024). In this study, 290 elderly patients with hip fractures were included, in which patients with 25OHD deficiency (<12 ng/ml) were used as the reference group. They observed a 56–64% increased rate of walking in patients who had 25OHD levels > 12 ng/ml at 30 days and 60 days after hip fracture surgery compared with 35% for patients able to walk 30 days postoperatively who had 25OHD levels < 12 ng/ml (Hao et al., 2020). It is important to note that only the preoperative 25OHD levels accurately reflect the patient's ability to walk after 30 days, and the postoperative vitamin D status is not related and should not be used to determine clinical or nutritional interventions. Holick (2020) releases a call for action, discussing the data collected by Hao, to establish guidelines which will assess vitamin D status as needed for patients with hip fracture. Holick suggests that "patients aged ≥50 y presenting with fractures, especially those with hip fracture, should be evaluated at intake for their vitamin D status. Consideration should be made to provide vitamin D supplementation if dietary/supplemental intake or blood concentrations of 25(OH)D suggest deficiency" (Holick, 2020).

Another randomized clinical trial administered by Scragg et al. (2017) provided a monthly high dose of vitamin D to 5,108 participants in order to determine if a relationship exists between increased vitamin D levels and cardiovascular disease prevention. This double-blind trial was placebo-controlled; participants were given an initial dose of 200,000 IU of vitamin D, and then each month after for a range of 2.5-4.2 years were given 100,000 IU of vitamin D (Scragg et al., 2017). Results showed that in a random sample of

Clinical conditions
– Differential diagnosis (i.e. hypercalcemia)
– Undernourished subjects
– Malabsorption syndromes (i.e. celiac disease, Chron's disease, radiation enteritis)
– Eating disorders (i.e. morbid obesity, anorexia and bulimia)
– Candidates for bariatric surgery
– Conditions associated with altered calcium, phosphorus or vitamin D metabolism (i.e. osteoporosis, rickets, renal disease, liver failure, multiple mieloma, sarcoidosis, hyper/hypoparathyroidism, liver and kidney transplants)
– Diseases related to low or null sun exposure (i.e. lupus, porphyria)
– Vitamin D-related inborn errors of metabolism
Therapeutic criteria
– Pharmacotherapy associated with increased vitamin D catabolism (i.e. antiseizure drugs, glucocorticoids)
– Treatment for AIDS and tuberculosis
– Monitorization of vitamin D treatment
Biochemical indicators
– Alterations of serum or urine levels of calcium and phosphorus
– Elevation of alkaline phosphatase (in the absence of altered liver enzymes or growth)
– Serum levels of parathyroid hormone out of the reference range (14–72 pg/mL)
– Previous (<6 months) serum values of 25-OH-vitamin D out of the reference interval (<37.5 or >160 nmol/L)

438 participants, cardiovascular disease occurred in 11.8% of patients who received vitamin D supplements and in 11.5% of patients who received placebos. This suggests that vitamin D administration does not prevent cardiovascular disease and should not be used for this purpose (Scragg et al., 2017).

Zhao et al. (2015) carried out a study within a primary care cohort the UK. Vitamin D results of 9,460 (74%) first tests and 3,263 (26%) retests were analyzed. Of the first-test results, 42% of patients were deficient. The authors noted a marked increase in Vitamin D testing over the six-year period of the study. However, a significant amount of the test requests were retests. The authors cautioned against over-testing for Vitamin D too soon, before serum levels could show adequate response: "A significant proportion of requests were retests. Despite guidelines recommending retesting after three to six months, 20% of retests were performed within three months. Our results suggest that retesting soon after intervention may not allow sufficient time for serum levels to respond. By contrast, retesting within four weeks of a large loading dose may give a false picture of over repletion" (Zhao et al., 2015).

Regarding pregnancy, vitamin D deficiency is common around the world and threatens fetal health and growth. Results from 203 Indonesian pregnant individuals who were followed from their first trimester until delivery showed astronomical vitamin D deficiency rates at approximately 75% (Yuniati et al., 2019). Data collected from these individuals included maternal demography, bloodwork to test ferritin levels, 25(OH) vitamin D results in their first trimester, and the final birthweight of the child after delivery. Final results did not show any association between ferritin, hemoglobin level, and vitamin D in either the first trimester of pregnancy or in the final birthweight of the neonates after delivery; however, the authors suggest that other unknown variables may be important and that nutritional supplementation during pregnancy is still vital (Yuniati et al., 2019).

Research has also been conducted on the association of 25(OH)D levels and SARS-CoV-2 infection. Ribeiro et al. (2021) conducted a retrospective cohort study on 1638 patients tested for SARS-CoV-2 infection and found that "previous insufficient 25(OH)D (<30ng/mL) concentration and high total cholesterol were associated with SARS-CoV-2 infection among adults >48 y in the study population." This may be attributable to the role that vitamin D serves in the immune system and its anti-viral activity through autophagy, as well as its high expression in cells of the lungs, thus rendering those with lower levels of 25(OH)D more susceptible to infection without these defenses (Ul Afshan et al., 2021).

Szerszeń et al. (2022) also investigated the possible correlation between the immunomodulatory effect of vitamin D and the incidence and progression of COVID. From a sample of 505 patients, they quantified serum 25OHD and analyzed each patient's COVID severity through the serum Vitamin Modified Early Warning Score (MEWS), "which includes respiratory rate, systolic blood pressure, heart rate, temperature and state of consciousness," along with the days spent in the intensive care unit. The results demonstrated that there was no difference in 25OHD concentration between those with and without COVID as determined by PCR and no correlation between serum 25OHD "in the COVID(+) group and the need for and time spend in the ICU as well as the MEWS score." However, multivariate analyses did show a positive correlation between the need for oxygen therapy and lower 25OHD concentration. This signifies the evolving role of vitamin D in and how low serum levels may aid in predicting more complicated treatment courses.

On the other hand, Javed et al. (2020) found that "high serum levels of vitamin D are associated with a lower risk of incidence and progression of [colorectal cancer]." This could make vitamin D testing crucial to identify possible future therapeutic modalities for patients with both low serum vitamin D and colorectal cancer. Like its mechanisms that hinder SARS-CoV-2 infection, such as being pro-apoptotic and anti-inflammatory, vitamin D has been shown to "decrease growth and differentiation of colon epithelial

cells." With more large-scale human trials, testing and treatment using vitamin D can become more widely applicable.

It is also known that decreased vitamin D levels are associated with inflammatory bowel disease (IBD), though the mechanisms have not been fully elucidated (Nielsen et al., 2019; Vernia et al., 2022). Studies further suggest that vitamin D supplementation may positively impact the course of IBD, highlighting the utility of vitamin D testing in this patient population. It has been suggested that a daily dose of 2000 IU correlates with improvements in IBD symptoms and patient quality of life (El Amrousy et al., 2021; Goulart & Barbalho, 2022).

Guidelines and Recommendations

The Endocrine Society (ES)

In 2011, the Endocrine Society recommended serum testing of 25-hydroxyvitamin D for evaluation of vitamin D status in individuals who are at risk of deficiency, including those with osteoporosis, obesity, or a history of falls. 1,25-dihydroxyvitamin D testing was not recommended for screening of at-risk individuals, due to its very short half-life in circulation, but is recommended for a few conditions in which formation of the 1,25-dihydroxy form may be impaired (Holick et al., 2011).

In 2024, the ES updated their guideline on Vitamin D testing and focused primarily on 25(OH)D serum testing without recommendations on 1,25-dihodroxyvitamin D testing. The ES reiterated the lack of clinical trial evidence supporting routine screening for 25(OH)D and used this evidence to reaffirm a position against routine screening of Vitamin D in the general population. "No clinical trial evidence was found to support routine screening for 25(OH)D in the general population, nor in those with obesity or dark complexion, and there was no clear evidence defining the optimal target level of 25(OH)D required for disease prevention in the populations considered; thus, the panel suggests against routine 25(OH)D testing in all populations considered." One notable difference between the 2011 and 2024 guideline is the changed position on screening Vitamin D in adults with obesity.

Pertaining to Vitamin D testing, the ES recommends the following:

1. "In the general adult population younger than age 50 years, we suggest against routine 25(OH)D testing."
2. "In the general population aged 50 to 74 years, we suggest against routine 25(OH)D testing."
3. "In the general population aged 75 years and older, we suggest against routine testing for 25(OH)D levels."
4. "During pregnancy, we suggest against routine 25(OH)D testing."
5. "In healthy adults, we suggest against routine screening for 25(OH)D levels."
6. "In adults with dark complexion, we suggest against routine screening for 25(OH)D levels."
7. "In adults with obesity, we suggest against routine screening for 25(OH)D levels" (Demay et al., 2024).

Institute of Medicine (IOM)

After an extensive evaluation of published studies and testimony from investigators, the Institute of Medicine determined that supplementation with vitamin D is appropriate; however, guidelines regarding the use of serum markers of vitamin D status for medical management of individual patients and for screening were beyond the scope of the Committee's charge, and evidence-based consensus guidelines are not available (Ross et al., 2011).

National Health Service (NHS) Clinical Commissioning Group

In "Guidelines for the Treatment of Vitamin D Deficiency and Insufficiency" the UK National Health Service notes that re-testing for Vitamin D levels before three months of supplementation is not advised as "vitamin D has a relatively long half-life, levels will take approximately three months to reach steady state after loading dose or maintenance treatment" (NHS, 2016).

Royal Osteoporosis Society (ROS)

The Royal Osteoporosis Society (formerly known as the National Osteoporosis Society) recommends the measurement of serum 25 (OH) vitamin D (25OHD) to estimate vitamin D status in the following clinical scenarios: bone diseases that may be improved with vitamin D treatment; bone diseases, prior to specific treatment where correcting vitamin D deficiency is appropriate; musculoskeletal symptoms that could be attributed to vitamin D deficiency. The guideline also states that routine vitamin D testing is unnecessary where vitamin D supplementation with an oral antiresorptive treatment is already planned and sets the following serum 25OHD thresholds: <25 nmol/l is deficient; 25-50 nmol/l may be inadequate in some people; >50 nmol/l is sufficient for almost the whole population (Royal Osteoporosis Society, 2020).

American College of Obstetricians and Gynecologists (ACOG)

In a statement on gynecologic care for adolescents and individuals with eating disorders, ACOG specified that in patients with low bone mineral density (BMD), "A patient's 25-hydroxy vitamin D level should be checked and, if less than 30 ng per mL, the patient should be given supplementation for 6–8 weeks in the form of 2,000 international units daily or 50,000 international units weekly" (Wassenaar et al., 2018). Reaffirmed in 2021.

Concerning screening for vitamin D deficiency, ACOG states that there is insufficient support currently to recommend screening for all pregnant individuals for vitamin D deficiency but that "maternal serum 25-hydroxyvitamin D levels can be considered and should be interpreted in the context of the individual clinical circumstance" (ACOG, 2011). Additionally, ACOG mentions that, while there is no broad consensus on the ideal vitamin D level to maintain optimal health, most guidelines agree that a serum level of at least 20 ng/mL (50 nmol/L) is needed to avoid bone problems. Reaffirmed in 2021 (ACOG, 2011).

United States Preventive Services Task Force (USPSTF)

The USPSTF published their recommendation concerning screening of vitamin D deficiency in asymptomatic community-dwelling, nonpregnant adults in 2021. "The USPSTF concludes that the current evidence is insufficient to assess the balance of benefits and harms of screening for vitamin D deficiency in asymptomatic adults" (I statement) (USPSTF, 2021).

American Association of Clinical Endocrinologists, The Obesity Society, and American Society for Metabolic and Bariatric Surgery

For patients undergoing Rouxen-Y gastric bypass (RYGB), sleeve gastrectomy, or biliopancreatic diversion either with or without duodenal switch (BPD/DS), a baseline evaluation for vitamin D deficiency and a postoperative evaluation is recommended (Mechanick et al., 2019).

American Association of Clinical Endocrinologists (AACE) / American College of Endocrinology (ACE)

The 2020 guideline addressed fundamental measures for bone health for the diagnosis and treatment of postmenopausal osteoporosis. The following statements apply to Vitamin D:

1. "Measure serum 25-hydroxyvitamin D (25[OH]D) in patients who are at risk for vitamin D insufficiency, particularly those with osteoporosis (Grade B; BEL 2)."

2. "Maintain serum 25-hydroxyvitamin D (25[OH]D) ≥30 ng/mL in patients with osteoporosis (preferable range, 30 to 50 ng/mL) (Grade A; BEL 1)."

3. "Supplement with vitamin D3 if needed, with a daily dose of 1,000 to 2,000 international units (IU) typically required to maintain an optimal serum 25(OH)D level (Grade A; BEL 1)."

4. "Higher doses of vitamin D3 may be necessary in patients with present factors such as obesity, malabsorption, and older age (Grade A; BEL 1)" (Camacho et al., 2020).

American Academy of Pediatrics (AAP)

"Evidence is insufficient to recommend universal screening for vitamin D deficiency… In the absence of evidence supporting the role of screening healthy individuals at risk for vitamin D deficiency in reducing fracture risk and the potential costs involved, the present AAP report advises screening for vitamin D deficiency only in children and adolescents with conditions associated with reduced bone mass and/or recurrent low-impact fractures. More evidence is needed before recommendations can be made regarding screening of healthy black and Hispanic children or children with obesity. The recommended screening is measuring serum 25-OH-D concentration, and it is important to be sure this test is chosen instead of measurement of the 1,25-OH_2-D concentration, which has little, if any, predictive value related to bone health" (Golden & Abrams, 2014).

Through the Choosing Wisely initiative, the AAP advises against routine vitamin D screening in otherwise healthy children, including those who are overweight or obese. Current evidence does not support the necessity of such screening, aligning with global recommendations against population-based screening for vitamin D deficiency. Instead, the AAFP recommends vitamin D supplements for children with insufficient dietary intake (AAP, 2022)

Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

A search of the FDA Device database on September 13, 2023, for "vitamin D" yielded 43 results. Additionally, many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). As an LDT, the U. S. Food and Drug Administration has not approved or cleared this test; however, FDA clearance or approval is not currently required for clinical use.

Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
82306	Vitamin D; 25 hydroxy, includes fraction(s), if performed
82652	Vitamin D; 1, 25 dihydroxy, includes fraction(s), if performed
0038U	Vitamin D, 25 hydroxy D2 and D3, by LC-MS/MS, serum microsample, quantitative Proprietary test: Sensieva™ Droplet 25OH Vitamin D2/D3 Microvolume LC/MS Assay Lab/Manufacturer: InSource Diagnostics

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

Evidence-based Scientific References

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Revision History

Revision Date	Summary of Changes
12/04/2024	Reviewed and Updated: Updated background, guidelines, and evidence-based scientific references. Literature review necessitated the following changes in coverage criteria: Edited CC3 for clarity and consistency. Removed Note 1 point S: "S. Obesity" Removed "medical necessity" language from Notes 1 and 2.